An-Najah National University

Faculty of Graduate Studies

BIOLOGICAL AND GENETICAL INVESTIGATION FOR CONSERVATION OF PALESTINIAN *IRIS HAYNEI* OF FAQQUA

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This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Environmental Sciences, Faculty of Graduate Studies, An-Najah National University, Nablus - Palestine.

2022

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Dedication

I dedicate my research (dissertation) to my beloved mother who followed me in each step of my life. I also dedicate this work to all mothers of my homeland Palestine. Thanks a million, to my father and family who always support me and stand by my side in all circumstances. My dedication continues to my friends and teachers who have never left my side throughout the process and are very special.

Acknowledgements

Many individuals, for too numerous to mention, have helped us to develop and refine the ideas and materials of this research. I would like to thank Prof. Raed Alkowni a senior supervisor from An-Najah National University who supported me in every single step of my study. I also would like to thank Dr. Rula JadaAllah as a second supervisor for the Arab American University. I also would like to thank the teachers Mahmoud Arda, Dr.Samir Jarrar as an external examiner, Dr.Hiba AL-faris as an internal examiner and Dr. Mohammad Jaber from Palestinian Technical University – Kadoorie.

Teachers Hiba-Al-ardah , Asma Kmail from Arab American University. Dr. Naser Shooli ,Thanks to the Wild Life Society the financier of this research, Teacher Ahmad Al-Omeri (A researcher in the science of Plants) and Teacher Monir Salah from Faqqua.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

BIOLOGICAL AND GENETICAL INVESTIGATION FOR CONSERVATION OF PALESTINIAN *IRIS HAYNEI* OF FAQQUA

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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BIOLOGICAL AND GENETICAL INVESTIGATION FOR CONSERVATION OF PALESTINIAN *IRIS HAYNEI* OF FAQQUA

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Abstract

Background:In Palestine, one of the symbolic flowering plants is *Iris haynei*. It is called "royal iris" and it's limited to the northeast of West Bank, particularly the village of Faqqua.

Aim:The main aim of this study was to better understand the current status of *Iris haynei* population to ensure their continuity in the Palestinian ecosystems.

Materials and method:Field surveys were conducted for some species to localize every plant on site and to check the fructification period (*in-situ* techniques). Past transplantation was monitored on-site and manual pollination was conducted and tested. On the other hand, seeds of the selected species were collected for the seed bank for the development of germination protocols (*ex-situ* techniques). Finally, after the lab studies have been conducted, the plants generated are ready to reinforce their original populations in their natural habitat (*in-situ* techniques). Besides, this study investigated the morphological and genetic variations of the planted Iris. For that, several *Iris haynei* species were grown in Faqqua Palestine and experiments were done including seed germination, genetic variation and site environmental scanning.

Results: In lab seed germination, 50% of seeds have been germinated in the presence of Gibberellic acid, while without hormone 34%. In the field, in the first year, none of the seeds have grown, while in the second year, 25% have grown. In" this study, the RAPD method was used for genotypes identification and characterization of "three *Iris haynei* colors (yellow, purple and pink). Five out of ten primers were tested for their ability to generate polymorphism from selected *Iris haynei* using RAPD-PCR, and the obtained data were analyzed. The similarity value analysis was 0.46 between (yellow, purple) Irises, while the similarity value between (yellow, pink) was 0.33. By this study, genetic diversities of studied were *Iris haynei* colored species ascertained successfully using RAPD markers, concluding that it could be a useful tool for identifying *Iris* species.

Keywords: ecosystem; *Iris haynei*; royal iris, RAPD.

Chapter One
Background And Introduction

There are between 340,000 and 390,000 species of vascular plants known to science [1], [2], and on average, 2,000 new species are discovered every year according to International Plant Names Index (https://www.ipni.org) / (accessed on:20-10-2022). Regarding ecology, distribution, dangers, and possible advantages, many of these plant species are little understood. A small percentage (less than 10%) of them have been evaluated for the IUCN Red List, with a significant preference for trees and species that are viewed as vulnerable [3] More than one in five of the 1,000 plant species that made up the study's sample were found to be in danger of going extinct (Brummitt et al. 2015). More than one in five of the 1,000 plant species that made up the study's sample were found to be in danger of going extinct [4]. The rate of plant extinctions now is up to 500 times higher than it was in the pre-industrial era [5] . Because of our limited knowledge of plant species, we are presently in a position where a significant number of plant species are threatened with extinction. While new species are constantly being discovered, some are also becoming extinct. Unfortunately, many more species are disappearing into oblivion without our knowledge or even our knowledge of them.

All biological study is built on the variety of organisms, but taxonomic expertise is needed to identify new species and define existing ones. Only 0.01% of the estimated 10-15 million species can be critically identified by even the most skilled taxonomists [6], [7]. At the 1992 Rio Earth Summit, the Convention on Biological Diversity (CBD) acknowledged this difficulty and, at its fifth Conference of Parties a few years later, launched the Global Taxonomy Initiative (GTI) (CBD COP5 1996). The GTI was established to lessen the taxonomic barrier and seeks to promote taxonomy while addressing knowledge and skill gaps. The knowledge gaps in our taxonomic system (including those connected to genetic systems), the lack of qualified taxonomists and curators, and the effects these deficiencies have on our capacity to preserve, utilize, and distribute the benefits of our biological diversity make up the taxonomic impediment. It is necessary to accelerate taxonomy beyond conventional morphology-based methodologies and further integrate DNA-based approaches in order to meet the Aichi Biodiversity Targets, the Sustainable Development Goals, and to contribute to the post-2020 Global Biodiversity Framework. The loss of biodiversity is occurring more quickly than the rate at which we can identify and characterize new taxa because the worldwide scientific community lacks the knowledge and continuity to recognize every species variety [8]–[11]. A demanding and time-consuming procedure, species description can be made more efficient via open data sharing and integrated taxonomy [12]. According to [7], there are four important limits to morphological species identification phenotypic plasticity and genetic variability in the characters used for species recognition can result in incorrect identifications, morphologically cryptic taxa, which are common in many groups, can be overlooked, morphological keys are frequently effective only for a particular life stage or gender, and many individuals cannot be identified; and modern interactive keys represent a significant advance in the field of species identification.

The use of DNA-based species identification, also known as molecular identification, enables the precise identification of species from trace fragments such as pollen [13], [14], the detection of substitution in herbal pharmaceuticals [15], [16] the authentication of sustainable tropical timber [17], the monitoring of invasive alien species , and the discovery of illegal. We can now discover and identify species in environments and conditions that were inconceivable just a few decades ago, or even in 2020, thanks to advancements in molecular identification [18]. In particular, fungi and insects have seen an increase in the number of "dark taxa," or species identified only from DNA sequences and lacking a physical counterpart and identification for morphological description [19]–[21]. Dark species provide a problem for taxonomy (Page 2016, but they also show how molecular evaluations of biodiversity may outpace and advance traditional taxonomy). In order to overcome our global taxonomic barrier and enable systematics to more significantly contribute to the CBD post-2020 Global Biodiversity Framework, it is essential that species detection and discovery accelerate. Traditional taxonomy is required for the actual description and naming of newly found taxa, which should be accomplished by fusing morphology and DNA. However, given that tropical rainforests—important ecosystems that house megadiversity and undiscovered species—are being lost at rates of millions of hectares per year [22], it is more important to quickly identify the biodiversity that must be preserved than to give each taxon a name. We will be able to play a significant part in recognizing that biodiversity thanks to the present revolution in molecular identification.

As part of the Mediterranean region, Palestine is considered one of the important plant diversity hotspot reservoirs on a global scale [23]. Its geographical location is at the confluence of the three continents—Africa, Asia, and Europe distinguished it as a pathway for spreading flora between these three continents. It enjoys rich biodiversity despite having a tiny area compared to other countries in the region [2]. Palestine (West Bank and Gaza) is characterized by a great topography and climate variations, which are directly reflected by the distribution and diversification of agricultural, topography and biogeographic patterns. Based on the geomorphology, West Bank is divided into "diverse habitats covering five ecological zones: Central Highlands" (where most of the population lives)," Semi-coastal Region, Eastern Slopes, Jordan Rift Valley, and Coastal Regions" [24]. This diversity in natural geography, consisting of desert, mountainous, and flattened agricultural areas, directly influences Westbank's ecosystems and biodiversity, making this tiny area a natural museum of various organisms such as non-flowering, wild-flowering flora and other living creatures which are already correlated with the "five Phytogeographical" regions: "Coastal, Mediterranean, Irano-Turanian, Saharo-Arabian, and Sudanese/Ethiopian" [25] (Figure1).

Figure 1

Phytogeographical regions of Palestine Modified from (UNEP (2020)

Biodiversity in Palestine is threatened by many risks, including habitat destruction brought on by uncontrolled urban growth, resource overuse, overgrazing, drought and desertification, invasive alien species, and pollution. According to a study conducted by Kondraskov, biodiversity conservation in Palestine faces many serious obstacles and limits that are to blame for Palestine's vulnerable biodiversity, making it a hotspot of biodiversity in the region and a component of a larger hotspot, the Mediterranean [26]

These obstacles can be summarized as follows:

1- The lack of knowledge and the absence of a well-established plan for land use has resulted in overlapping and competing land uses as well as the development of real-world conditions that may obstruct future attempts to manage this scenario.

2- The protracted Israeli occupying force places a lot of strain on the Palestinian environment.

3- The incorrect use of lands due to geography and climate and the lack of systematic planning.

"In Palestine (West Bank and Gaza), about 2780 plant taxa were recorded as native or naturalized"; within these species, about 800 were rare plants, and from "native taxa, 162 were recorded as endemics" [27]. A flower known as Royal Iris [28], [29] botanical name "*Iris haynei"*, is one of the numerous flora and flowers that are somewhat threatened [30], [31]. This blossom is a prized-unique and lovely blossom in Palestine and in 2016 has been declared the national flower of the State of Palestine by the Palestinian Environment Quality Authority [32]. There are five types of Royal Irises that grow naturally in Palestine, the most important of which is the Iris of Nablus "*Iris ortetii*, al shefa flower "*Iris atrofusca*" and " *Iris haynei* " or Faqqua Iris [30].

Figure 2

Types of Royal Irises in Palestine, from the left, Iris ortetii, Iris haynei, and Iris atrofusca

All types of Royal Iris in Palestine "are included in the International Union for Conservation of Nature (IUCN) red list of threatened" plants, which gives them a high priority for Protection and preservation measures. In recent years populations of different Royal Iris varieties have witnessed a sharp decrease in the number and the destruction of their natural habitats. This causes the loss of these very important genetic resources of these varieties, and operations of preserving Royal Iris species are needed. Regrarding, Iris seeds need a long time (about two years) and special conditions to germinate, Rhizomes as a propagating material are a good way to preserve Irises genetic germplasm since sexual reproduction by seeds is a time consumer and germination percentage is very low [30], [33].

Iris haynie is found locally in the Marj Ibn Amer to Bisan mountain ranges, the eastern Jordan Rift Valley foothills, and the slopes of the mountains east of Jerusalem and primarily in sunny arid Batha on rocky-stony slopes in the eastern parts of the Gilboa ridge, Between Arabuna village and Deir Ghazaleh, as well as from the southern borders of Jalboun village to the village of Faqqua. This region's ancient boundaries were the fields of Bisan and Jabal Tabor, and it has a view of the Nazareth highlands. It contains Deir Abu Daif, Al-Mughayir, Zababda, and nearby villages and cities. This intensive area of Royal Iris fields is about 20–25 square kilometers making Palestine the only country in the world with such a dense field, which is located inside the boundaries of the hamlet and mountains of Faqqua. Irises from the Faqqua Mountains flourish due to their unique climate, where they grow in a region known for its natural diversity, picturesque landscape, fertile soil and precipitation of 500 mm, and appealing rocks. Hills in that area, the tallest in the eastern half of the Marj Ibn Amer highlands, rising 450 meters above sea level, make it an amazing exhibition of the region's natural topography [6]. Royal Iris Relatively bright flowers characterize the Gilboa population; towards the south, to the east of Nablus, the flower color gradually darkens, where there are transitional populations between *Iris haynei* and *Iris atrofusca* and *Iris ortetii* and identifying these populations without sharp differences between the three species is quite difficult. In addition, there are many threats to the Iris like agricultural practices (ploughing and using herbicides), destruction of natural areas, Urban encroachment, land reclamation, domestic and non-domestic tours resulting in a sharp diminishing population of *Iris haynei* and the likely extinction risk in the upcoming years, which led to designate it as a threatened species by Environmental Quality Authority and anticipated putting it within red list according to the standards of the (IUCN). Therefore, developing and evaluating *in-situ* and *ex-situ* techniques are necessary for conserving *Iris haynei* plants. Accordingly, the general objective of this study is to gain a deeper understanding of the current situation of *Iris haynei* populations in order to secure their survival in the Palestinian ecosystems and the state of their diversity in Faqqua.

The specific objectives are:

1- Identifying the distribution, population, and dynamic of the plant *Iris haynei*.
2- Compute the genotype (genetic variations using DNA fingerprint technology) and phenotype (morphological variations of the planted iris) of *Iris haynei*.
3- Conducting a set of seed germination experiments of *Iris haynei* seeds in order to multiply and conserve its communities.

Chapter Tow
Literature Review

2.1 Biology of Irises

Iriss (genus *Iris*) are perennial monocotyledonous geophytes. The genus belongs to the Iridaceae family and consists of "six subgenera: *Nepalensis Dykes*, *Xiphium* (Miller) Spach, *Scorpiris* Spach, *Hermodactyloides* Spach, *Iris L*. and Limniris Tausch "[34]. According to Mouterde subgenera: *Hermodactyloides*, *Iris*, *Limniris*, and *Scorpiris,* with more than 300 Iris species globally [35]. "Irises have been known worldwide for centuries for their beautiful flowers, which hold many mythological, artistic, and historical evocations. Due to the wide spectrum of flower colors in Nature; the name iris is derived from the Greek word for rainbow" [36]. Genus Iris plants originated in the Northern Hemisphere's temperate regions and are cultivated worldwide [37]. The "subgenus *Iris* is represented by two sections: *Iris L*. with two taxa and *Oncocyclus* (Siemssen) Baker with seven taxa, while the subgenus *Limniris* is represented by two taxa, the subgenus *Hermodactyloides* with one taxon and subgenus *Scorpiris* with two taxa" [34]. *Oncocyclus* includes "33-45 taxa, considering subspecies, forms and varieties", all of "which are regional endemics" [38]. *Oncocyclus* species grow in small disjunctive populations separated by short geographical distances as scattered populations. They are locally found across rocky hillsides, steppes, and deserts from the north Caucasus south to Iran, Turkey, Syria, and other countries on the eastern shores of the Mediterranean Sea as well as Palestine [39]. According to Wilson *et al*. (2016), the "Eastern Mediterranean region is an important area of diversification", while the Caucasus region considers the ancestral area for the *Oncocyclus* section [28].

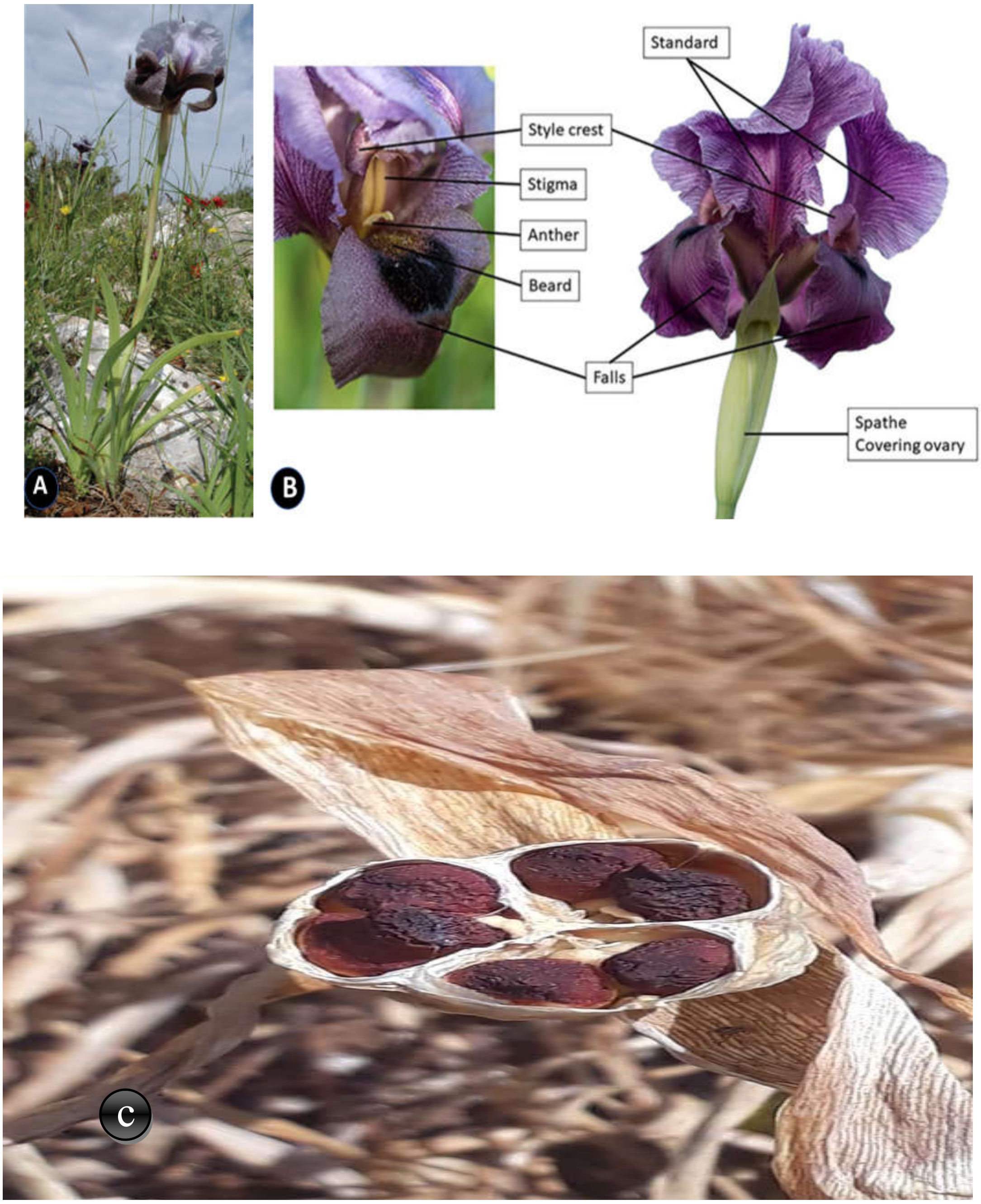
From a taxonomic point of view, plants of the *Oncocyclus* section are particularly challenging within the Iridaceae family [34]. Sympatric distribution and interfertility among Oncocyclus species result in a large number of hybrids, often recognized as full species with minor differences in plant size, leaf shape and flower color, which are used for taxonomic identification [34], [39], [40].

Irises have short, knobby rhizomes, occasionally with stem-like runner stolons that produce fans of unifacial basal large leaves that are typically falcate and glaucous, geophytes plants, meaning they have an underground store of nutrients, such as a bulb or rhizome [41]. Species of sect. *Oncocyclus* are easily recognized by their flower coloration and morphology (Figure 3). Inflorescences have a single spherical flower typically held well above their leaves. They produce tertiary symmetrical flowers with three petals on the outside called "Falls" that are largely dilated and three petals on the inside called "Standards" that are often erected. The flower stigmata is located behind the flap of the petaloid-style

branch, and the flower's anthers are inserted at the base of the labellas. The ovaries are located just below the flower part, specifically beneath the stylar column (Figure 3). Petals and sepals are large, usually darkly veined and/or sprinkled with dark markings on a lighter background, each sepal has a prominent roundish spot near the base of its free portion called a signal spot. Section *Oncocyclus* species also have a conspicuous white or cream-colored aril that is sometimes larger than the seed. The chromosome number of *Oncocyclus* species is 2n = 20 [42].

Figure 3

Main parts of Oncocyclus species flower, Iris haynie seeds



2.2 plant molecular identification

2.2.1 Distribution of plant DNA in the cell

The majority of DNA extraction techniques remove all cellular DNA. In some experimental situations, it may also be desirable to target either nuclear DNA or DNA found in the genomes of organelles (in plants: mitochondria and plastids). The genomes of organelles are substantially smaller than any nuclear genome in a plant.

A plastid genome, on the other hand, has a relatively stable genomic structure and is typically approximately 150 Kbp in size. Examples of plastid genomes include those found in chloroplasts of leaves and amyloplasts of cereal grains [43]. They include the plant DNA barcode genes matK, rbcL, and trnH-psbA and have a high enough rate of substitution to be effective as a molecular tool in investigations at many phylogenetic levels, including population-level and phylogeographical ones [44]. Plastids are found in large copy numbers in DNA extracts from plants because they have several copies of their plastid genomes and numerous plastids per cell. They are therefore particularly useful for sequencing from entire DNA samples, using, for instance, Nuclear genome sizes are quite varied, especially in angiosperms, with the mean and modal 1C (the amount of DNA in an unreplicated gametic nucleus) both being at about 5 pg/Gbp [45]. Most monocots have the biggest genomes, especially those in the Liliaceae and Melanthiaceae, including the record-holder Paris japonica, which has almost 150 Gbp of DNA [46]. Carnivorous members of the genus Genlisea have been shown to have the shortest genomes, such as the 61 Mbp genome of *G. tuberosa* [46].Only lately have molecular techniques for high-throughput DNA sequencing, particularly those that simplify genomic complexity, enabled genome-wide investigations on the biggest plant genomes [47]. Hi-C technology is one example of how methods for high quality (chromosome-level) assembly of huge plant genomes have improved [48], [49].

2.2.2 Experimental history and main principles of DNA extractions

While researching proteins from leukocyte nuclei, Swiss physician Friedrich Miescher unintentionally isolated the first piece of DNA in 1869 [50]. When acid was added to a solution, a material precipitated out of it, but when an alkaline solution was added, it dissolved again. This precipitant was dubbed "nuclein" by him. The fundamental objective of DNA isolation techniques nowadays is to separate intact DNA from other plant cellular components while minimizing DNA degradation, even if contemporary protocols are far more developed than the very first attempts. In addition to a cell membrane, plants also have a hard cell wall consisting of cellulose and other substances like lignin. This demands a powerful initial step for plant DNA extraction that breaks down cell walls and disintegrates the structure of the plant tissue. This might entail flash freezing the tissue with liquid nitrogen, followed by grinding with a pestle and mortar in a low-throughput environment (or for samples that are harder to disrupt). Machines that disturb tissue can be used for better sample throughput. The ground material should then be moved on to the chemical phases of the procedure right away, which require rupturing the cell membrane to release the lysate that contains the soluble DNA. Next, it is separated from other insoluble material and cell waste. The leftover material, which may comprise soluble proteins, nucleic acids, and tiny molecular metabolites, is then separated into DNA molecules using a variety of techniques [51]. Common endogenous contaminants in DNA extractions include cellulose and lignin generated from the cell wall, as well as polysaccharides, polyphenols, tannins, and other secondary metabolites (especially frequent in medicinal plants). These substances must be isolated and eliminated as much as possible since they could prevent subsequent laboratory processes and result in inferior sequencing [52]. However, it can frequently be difficult to extract enough large amounts of high-quality and high-purity DNA from plants.

To extract DNA from plant material of various origins, several techniques and processes have been devised [53]–[55]. It is frequently necessary to optimize a process or combine it with another in order to get high-quality DNA from a particular plant material. The source tissue, the material's age, and the concentration of metabolites in the plant are just a few of the variables that will affect how the best separation method is refined.

A significant advancement in plant DNA extraction procedures developed by Doyle and Doyle [56]. Small quantities of plant tissues are used in this approach to extract DNA using the cationic detergent CTAB. This was a much-appreciated replacement for the time-consuming, costly, and dangerous caesium chloride ethidium bromide density gradient centrifugation method [57]. Due to its adaptability and scalability, notably in the amount of detergents used.

2.2.3 Storing and preparing plant material for DNA extraction

Plant material

Healthy plant tissues such as leaves, flowers, buds, seeds, roots, bark, and even spines may all be used to harvest DNA. For herbaceous plants in particular, young leaf tissue is the recommended starting material [58]. and fresh leaf tissue often generates significant quantities of good-quality DNA [59]. But the kind of substance employed for DNA extraction relies on its accessibility. A practical approach can be necessary depending on availability owing to seasonal change and plant life cycles. During sample collection and storage, some plant tissues (such as roots and stems), clades (such as ferns; [60]. and morphological traits (such as succulence; [61]. provide unique obstacles that call for specialized processing techniques.

Any plant material that is to be used for high-quality DNA extraction must be properly processed, dried quickly (without using excessive heat treatment), and maintained in a dark, dry environment to prevent DNA deterioration. Endogenous nucleases released during cellular lysis are what induce DNA breakdown before extraction; these nucleases may be enhanced by environmental conditions like heat and humidity [62].

The available plant material dictates the extraction technique. A 1 cm2 piece of herbaceous leaf tissue will be sufficient for a single extraction for the majority of kit- and CTAB-based methods. To verify the accuracy of sequencing results against the specifics of the lab's extraction process and to identify the causes of variation between samples, careful laboratory notes of the material used are essential. These notes should include provenance information, sample weight, and the extraction date. In accordance with some protocols, weighted tissue can be directly added to a 1.5 ml tube that has been labeled with a special number, laboratory code, and other details in preparation for the DNA extraction procedure.

Silica drying

Plant material that has been dried and preserved in silica gel, such as specimens kept in tissue banks expressly for the purpose of DNA extraction, is frequently a reliable source of high-quality DNA. A desiccant, such as silica gel (silicon dioxide xerogel), dries out plant tissue by removing moisture from the air. When the silica is saturated, indicator silica gel crystals change color, indicating that it is time to renew or replace the silica gel. These crystals can be used with silica gel without indicators.

Because it is less expensive and more practical than using liquid nitrogen or lyophilization to dry fresh plant material for DNA extraction, silica gel is frequently used, especially when processing tissue on the spot. The suggested minimal ratio of plant material to silica for efficiently preserving the DNA in plant tissue is 1:10 [63]. To speed up desiccation, the volume of tissue should be decreased and sliced into smaller pieces if the plant material being gathered is mucilaginous, thick, or hardy. This will allow the desiccant to come into touch with the cut surface of the plant material. The quantity of silica required and how frequently it must be replenished depend on the climate in which plant material is gathered; a humid environment calls for regular desiccant replacements. A breathable material, such as a folded tea bag or coffee filter in a sealed container containing silica gel, provides an alternative to directly storing tissue samples in individual, sealed plastic bags containing silica gel. The latter approach is advised to reduce sample contamination and sample powdering caused by friction with the silica gel beads, which makes it more challenging to remove the tissue from the container in the future. Each sample should have two labels: one on the outside

Freezing

One method is to freeze plant tissue until it is required for DNA extraction, preferably at -80 °C, although if the sample is well packed, it may also be kept at -20 °C in a typical laboratory freezer. An alternative is to quickly freeze the substance with liquid nitrogen. High-quality DNA extractions may be made from the resulting swiftly frozen material, although liquid nitrogen isn't always viable because of handling issues and expense [64]. Additionally, it's best to avoid repeatedly freezing and thawing plant tissue because doing so can harm the DNA, organelles, and cells of the plants [65]. Therefore, it is advised that frozen plant material only thaw once, just prior to DNA extraction.

Lyophilization

Tissue that has been lyophilized (or frozen dried), such as leaves and roots, can be used to extract high-quality DNA [66]. When new material cannot be utilized right away or is not accessible, this approach, which was developed in the 1960s, is still employed. Lyophilized plant material can provide high-quality DNA when combined with the right extraction method [67]. Plant tissue is kept at low pressures (0.1 mbar) and temperatures (-50 °C) during lyophilization, which causes the water in plant cells to sublimate. Usually, a condenser is installed, capturing the vaporized water as ice. The plant tissues can be withdrawn from the lyophilizer once all the water has been removed from the plant material (usually accomplished in a few hours or over night). Next, the lyophilizer is brought to atmospheric conditions. It is advisable to move on with mechanical tissue disruption right away in order to prevent ambient moisture. The sample can instead be kept in silica gel before being used again.

2.2.4 DNA extraction protocols

- A DNA extraction methodology can be used after the plant material has been prepared by drying and/or freezing using one of the methods outlined above. Although there are other protocols available, the typical process entails the procedures listed below, which are covered in greater depth below:
- Weighing of plant tissue
- Mechanical disruption (grinding)
- (Optional) pre-treatment
- Extraction of nucleic acids from the cell
- DNA isolation and precipitation
- DNA purification

We place emphasis on the CTAB protocol due to its popularity, but also introduce other protocols that may be of interest to the reader.

General workflow for DNA extraction

Weighing plant tissue

An inadequate yield can be caused by starting with too little or too much plant tissue; too little will result in poor grinding, saturation of the reaction, and/or too much debris, all of which can hurt the ultimate yield. A buffer quantity that is five times the weight of the leaf tissue, such as 0.2 g of leaf tissue for 1 ml of buffer, makes for an effective beginning point [68].

Mechanical disruption (grinding) of plant material

For the chemical reagents in the next stages to work effectively, plant tissue must be finely crushed to a powder so that the cell walls are broken and the cell membranes are more exposed. Before weighing and grinding, it is advisable to remove hairs or wax from the plant tissue's surface. Since glue might interfere with the chemicals used for DNA extraction, great care should be taken to ensure that it is removed from herbarium specimens. To increase the friction and facilitate the breakdown of the tissue, sterilized sand can also be added; it will be separated later on in the DNA extraction technique. Manual grinding costs nothing but takes a lot of time and calls for a sterile mortar, pestle, and spatula for every sample. It is more effective to use a mechanical homogenizer, also known as a tissue lyser. Each sample-containing tube is given a steel ball bearing and shaken vigorously inside the device. This enables simultaneous disruption of numerous samples with little nucleic acid degradation. Due to the fact that each sample is treated in the tube in which it will remain for the remainder of the extraction process, it also minimizes material loss and the possibility of contamination. To maximize the disruption of especially hard or woody material, the sample tubes might be filled with different-sized metallic, ceramic, or silica beads. Before continuing with the treatment, metallic and ceramic beads must be taken out, however silica beads can be separated later on.

Optional pre-treatment

This procedure can be used as part of an optimization approach to improve the yield, quality, or purity of the DNA that is extracted. For instance, the modified STE-CTAB procedure might be utilized when high levels of polysaccharides and/or polyphenols in the plant material are a concern (as is the case, respectively, for succulent plants and plants under high stress conditions) [69]. The usual CTAB approach can then be used after the ground plant tissue has been washed up to three times with a Sucrose-Tris-EDTA (STE) buffer, which dissolves the majority of the polysaccharides and polyphenol. An alternate sorbitol-based pre-wash can be helpful in removing polyphenols and getting DNA with improved purity as a result [70].

Extraction of nucleic acids from the cell

The objective at this step is to release nucleic acids from the cell while minimizing the danger of nucleic acid deterioration and to start separating undesirable cellular substances from the DNA molecules.

Cetrimonium bromide (CTAB) extraction buffer, which must include the following ingredients, is the distinguishing feature of the most extensively used technique for DNA extraction from plants, which was first established by [55], [56].

- 2% w/v CTAB: a cationic detergent which, during DNA extraction, binds to the lipids in cell membranes, enhancing cell lysis, thus releasing intact nucleic acids from the nucleus and organelles
- 1.4 M NaCl: a salt which increases the ionic strength of the solution, which simultaneously induces plasmolysis, promotes separation of proteins from DNA, and aids in polysaccharide precipitation
- 100 mM Tris-HCl: a buffer (at pH ~8.0) which maintains the pH of the solution and stabilises the DNA by impeding degradation
- 20 mM EDTA (ethylenediaminetetraacetic acid): which protects the DNA by inhibiting the enzymatic activity of DNase and RNase (i.e., by chelating divalent cations, such as Mg^{2+} and Ca^{2+}, which are cofactors for these enzymes)
- 0.2% ß-mercaptoethanol: which denatures polyphenols and tannins (abundant in plants), rendering it possible to separate them from the DNA in subsequent steps

Each sample tube containing powdered plant tissue receives a dose of CTAB buffer, and the combination is then incubated for 15 to 60 minutes at 60 to 65 °C. You can carry out this in an incubator with automated shaking. As an alternative, the sample tubes can be manually shaken periodically.

Alternately, strategies utilizing an SDS buffer might be used [71]. NaCl, Tris-HCl, EDTA, and ß-mercaptoethanol are likewise present in the buffer formulation, but it additionally includes sodium acetate and sodium dodecyl sulphate (SDS), an anionic detergent used to break cellular membranes (NaCH3COO).

DNA isolation and precipitation

By using the different polarity of these molecules, this step aims to separate DNA from other molecules in the lysate. DNA precipitation from the solution happens next.

Phase separation using organic solvent(s) is the technology used in the CTAB process to separate hydrophilic molecules, such as DNA. The combination of CTAB and leaf tissue is treated with a 24:1 solution of chloroform-isoamyl alcohol (SEVAG buffer). To prevent inhalation, this solution must be prepared and applied to the sample tubes in a fume hood. It should be handled immediately to prevent evaporation during the job since it is also extremely volatile and evaporates fast. The DNA is concentrated in the clear top phase after centrifuging the mixture at room temperature (i.e., the aqueous phase). Without touching or disrupting the organic phase, which contains the chloroform along with lipids, proteins, and other cellular detritus, the supernatant is very carefully pipetted off and transferred to a fresh tube. RNase A and cold isopropanol are added to the supernatant to purify it, with the latter causing DNA to precipitate. The samples are then placed in a freezer at -20 °C, either overnight or for a number of days if maximal precipitation is desired but sample input is limited (at the cost of potential co-precipitation of salts).

Proteins and polysaccharides precipitate together with the SDS in the SDS procedure. The DNA is then precipitated using sodium acetate; when this substance is in solution, sodium ions (Na^+) neutralize the negative ions on the sugar phosphate backbone of DNA molecules, making the molecules less hydrophilic and more precipitation-friendly [72].

Both procedures culminate with centrifugation of the samples to promote the production of a DNA pellet, optionally followed by at least one wash with 70% ethanol and resuspension, preferably in 10 mM Tris-EDTA buffer (which serves to protect the DNA from damage, as explained in the CTAB buffer recipe above).

DNA purification

It is not perfect to isolate DNA at this point. Co-extraction of polysaccharides with identical molecular compositions is frequent because the extraction procedure includes phases that separate molecules by binding characteristics and molecular weight.

Additionally, the eluent may contain a variety of impurities, such as endogenous proteins, tannins, polysaccharides, and other compounds, as well as small amounts of substances that were introduced during the extraction process and precipitated salts. By acting as PCR inhibitors, these substances might adversely affect the DNA's usage in subsequent experiments, necessitating further purification of the DNA using other cleanup procedures.

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Solid Phase Reverse Immobilization (SPRI) beads are an option [73]. These beads are paramagnetic, which means that when they are in a magnetic field, they congregate. Their magnetite surface is covered with carboxyl molecules that, under particular chemical circumstances, may reversibly attach to DNA. The "crowding agent" in this case, polyethylene glycol (PEG), facilitates the binding of DNA to SPRI beads. This crowding agent's ratio to the DNA eluent is crucial because the higher the concentration, the stronger the DNA molecules' attraction to the beads will be, allowing for the binding of ever-smaller fragments with molecules of lower charge. Therefore, the first step is to select a ratio of DNA to SPRI beads, which are in solution with the crowding agent and salt (NaCl). Generally speaking, a ratio of 1:1 is suitable for DNA cleanup; however, for the retention of extremely small DNA fragments, this ratio can be raised to 2:1. The DNA will remain immobilized to the SPRI beads, which are drawn to the edges of the tube, close to the magnetic field, once the tube holding this combination is inserted onto a paramagnetic plate. At this stage, the supernatant in the tube, which includes any impurities and short nucleic acid residues, may be pipetted out. An elution buffer, such as 10 mM Tris-HCl, is added to the beads after two washes with an 80% ethanol solution to re-suspend the pure DNA.

Protocol optimization

Modifications can be made when a DNA extraction technique does not provide the desired quality or quantity of extracted DNA. Searching the scientific literature for techniques that have been used to comparable experimental goals or that have targeted the same taxonomic groupings is an effective method for this.

Understanding the biochemical activities and interactions of the CTAB protocol's components is a great place to start when figuring out what would need to be changed to assist improve the outcome. The concentration of NaCl must be at least 0.5 M for CTAB to bind to proteins and neutrally charged polysaccharides instead of nucleic acids and behave in accordance with the ionic strength of the solution. The concentration of NaCl that is most frequently used is 1.4 M. Higher NaCl concentrations may increase the quality of the final DNA when working with a plant group that contains a lot of polysaccharides.

It is occasionally possible to add other chemicals, such as N-Lauroylsarcosine (sarkosyl) buffer, to increase lysis (rupturing of the cell membrane) and lessen the activity of DNase or RNase enzymes. Protein denaturation can be accelerated by adding proteinase K. Additionally, the 24:1 chloroform-isoamyl alcohol solution's volume can be changed. Instead of using only chloroform, phenol can be used as an additional non-polar, organic solvent that is highly successful in denaturing proteins and can help to increase the final DNA yield [72]. However, it is quite dangerous and needs to be handled carefully.

Nearly all procedures contain EDTA and tris-HCl. Because ß-mercaptoethanol is hazardous, it should always be handled in a fume hood equipped with an extractor fan. For plant tissues with low levels of phenolic compounds, one may simply think about leaving this reagent out of the solution. It's crucial to remember that phenolic chemicals co-precipitate with DNA and can, thus, cause issues in later stages of DNA laboratory work. ß-mercaptoethanol can be substituted with less harmful substances like PVP (polyvinylpyrrolidone). PVP utilizes hydrogen bonding to bind to phenolic chemicals, which may then be separated from them following centrifugation[72]. PVP has been discovered to enhance DNA extraction from tissues like wood). PVP (polyvinylpolypyrrolidone), a substance that is similar to PVP but primarily differs from it in that it raises the pH of the extraction buffer, has also been discovered to boost the yield of DNA extracts [68]. The use of a 4-6 hour incubation at 45-55 °C or an overnight incubation is the final optimization step for more resistant plant tissues, which increases the yield of the extracted DNA.

2.3 Genetic Variation

"In plants, morphological characters have been investigated" in the context of variations of the "environmental gradients, such as latitude/longitude" [74]–[76], climate [75], [77] rainfall [78], soil and nutrients [79]. And it is common knowledge that the factors influencing genetic variability both within and between plant species differ significantly [80], [81]. "Allele distributions and gene flow within species ranges" are likely to be impacted by geographic "range, population size, successional status", and a variety of other characteristics (life form, mode of reproduction, mating system, and dispersal capacities) [82]. Additionally, a population's history (recent inception, the presence of bottlenecks, or range extension) may have a substantial impact on the population genetic structure [83].

Irises have been cultivated for over 400 years. It was introduced to Europe in the 15th century. Sausan is the Arabic word for Iris, and the plant may have been cultivated in Turkey before its introduction to Europe [28]. Despite the long history of studying the sect. *Oncocyclus*, its consistent recognition as a taxonomic entity, and the stability of species restriction within a taxonomic group, there have been a few hypotheses proposed about interspecific relationships. "Seven aggregates have been described within section *Oncocyclus* on the basis of the floral morphology, flower colour and the micromorphology of hairs at the entrance of the pollination tunnel" [39].

Regional studies on species diversity have utilized morphology [29], [40], [84]. Sapir *et al*. (2002) studied the morphological characteristics of the *Oncocyclus* to determine the relationships among 42 populations; they investigated the vegetative and productive characteristics. Rahimi *et al.* (2009) studied *Iris* landraces of Iran. Naderi et al. (2007), Arafe *et al.* (2002) studied different Palestinian species of *Iris* by morphological traits [35].

Despite the amazing advances in plant taxonomy based on morphological traits of plants, "the classification of *Iris* is indeed a difficult task to tackle; botanists and taxonomists are still far from reaching a consensus on this issue. This problem is certainly reflected through the different subgeneric and sectional classifications established based on morpho-anatomical features, and ecological and cytogenetic traits" [34]. *Iris* genus transition forms and the continuous Nature of morphological variation between genus species are characteristics of Iris, "more especially among royal irises" (*Iris* sect. *Oncocyclus*). This led to much confusion in attempts to distinguish species using morphological criteria [34].

Nucleic acid sequences have a variety of applications in the field of molecular biology. They are a valuable tool in many analytical and application techniques used in molecular biology, health, medicine (gene therapy, diagnostics, and recombinant protein expression), forensics, and food science. Some examples of these techniques include next-generation sequencing applications, genotyping with DNA fingerprinting, detection of pathogens, and forensic identification of biological samples and environmental samples contaminated with different biological entities [85], [86].

To be used as a diagnostic tool, the target nucleic acid sequence should be free of contaminants that inhibit PCR and other downstream applications. Such contaminants chemically or mechanically block or inhibit chemical and enzymatic reactions, including denaturation and hybridization of nucleic acids and other applications used in molecular biology methods. Pollutants can also degrade or modify nucleic acid. These include high-molecular substances, such as polysaccharides and polyphenols, and substances of lower molecular weight, such as pigments and secondary metabolisms [86].

DNA-based markers revealing polymorphisms at the DNA level are very useful tools in genetic studies and in the improvement of crop plants, and present numerous advantages over conventional phenotype based methods, they can be applied to a variety of purposes including DNA fingerprinting, genetic screening and chromosomal mapping" [87].

Genetic markers can be categorized into four kinds "(single-locus marker, dominant marker, co-dominant marker, and multilocus marker)" [88]. Restrictions fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA markers (RAPD), and amplified fragment length polymorphisms (AFLP) are among the DNA polymorphism assay methodologies [89]. Because it is so straightforward, RAPD has seen a significant growth in use as a "molecular marker for taxonomic and systematic investigations of plants, as well as in plant breeding and genetic diversity assessments [90]. RAPD markers are DNA segments that can be amplified randomly using any "nucleotide sequence (short primers usually about ten nucleotides length)". Many times, "only a small number of primers were required to detect polymorphism in a particular species". In fact, it may frequently be possible to discriminate between all of the examined types with just one primer [90].

(RAPD) markers was used to analysis of phylogenetic relationships within 12 Siberian *Iris* species [91]. Study conducted using RAPD has provided information regarding the genetic relationships between *I. haynei* and *I. atrafusca* [29], RAPD markers also used to determined the genetic variability of *I. setosa* populations [92]. "Analysis of seven *Iris* species-specific random amplified polymorphic DNA (RAPD) markers and two chloroplast DNA haplotypes are compared with the environmental gradients in a Louisiana Iris hybrid population. This study suggested that, at a very fine spatial scale, environment-dependent selection contributed to the genetic structuring of this hybrid zone" [93].

The "RAPD technique was successfully used in studies of the systematic relationships between five of the Far Eastern *Iris* species. A set of arbitrary primers (OPD-08, OPD-11, OPD-13 and OPB-12) suitable for inter-and intraspecific polymorphism analysis in *Iris* was followed; they gave stable, well-reproducible, species-specific patterns for Iris species with no variation between individuals from the same populations" [94].

Previous studies, based on the inclusion of representative species in phylogenetic analyses of the *Iris* genus , have determined that the *Oncocyclus* sect. is monophyletic [95], [96]. Phylogenetic studies based on plastid data of *Iris* have revealed a further understanding of subgeneric relationships and provide a framework to explore relationships within and among major clades completely. However, studies have been inadequate to resolve relationships in some newly diverged clades, such as sect. *Oncocyclus* (Siemssen) Baker in subg. *Iris* [96]. some subclades in subg. *Scorpiris* Spach, and ser. *Californicae* (Diels) G.H.M.Lawr. in subg. *Limniris* [97]. Even though most species are easy to recognize based on morphology [97]–[99].

2.3 Seed Germination

Seed germination is the fundamental process in which plant species grow from a single seed into a plant. Quality and effectiveness of germination influence both crop yield and quality later. A common example of seed germination is the sprouting of a seedling from an angiosperm or gymnosperm seed.

During the beginning stage of germination, the seeds take up water rapidly, resulting in swelling and softening of the seed coat at an optimum temperature. This stage is referred to as Imbibition. It starts the growth process and by activation of enzymes. The seed activates its internal physiology, starts to respire, produces proteins, and metabolizes the stored food. This is a lag phase of seed germination.

By rupturing the seed coat, a radicle emerges to form a primary root. The seed starts absorbing underground water. After emerging the radicle and the plumule, the shoot begins growing upwards.

In the final stage of seed germination, the cell of the seeds become metabolically active, elongates and divides to give rise to the seedling.

Species of *Iris*, *Oncocyclus* sec. are characterized by seeds with hard seed coats that "developed by dehydration during the ripening process" at the end of the Spring during the first summer, at which the dispersed seeds dry and shrink [42]. These species grow in herbaceous scattered plant communities and experience semi or arid climates, in such environments, "precipitation is low and unpredictable", and slow and sporadic germination is considered to be an adaptive strategy over many years is traditionally "increasing the proportion of successfully established seedlings by spreading the risk of germination failure "[33]. These seeds have a slow germination rate and strong dormancy in their local habitats. The germination rate is up to 15% in the first year and usually no more than 30% in the following few years, while the remaining "seeds germinate throughout the subsequent 5–6 years" [100]. And this period may extend lasting up to 20 years after ripening [33].

Blumenthal *et al*. (1986) "showed that the germination rate in *Oncocyclus* is species-specific" related [33], for example, in *I. lortetii* Barbey, only 1% of seeds germinate "in the first year while in *I. atropurpurea* Dinsmore the majority of seeds" the germination rate reach 60% in the first year [101].

All Palestinian Iris species of the sect. *Oncocyclus* are rare and endangered. Currently, many natural populations of these species" are decreasing in numbers and threaten with "being destroyed, causing the possible loss of unique genetic germplasm" with high priority for conservation [30], [42]. Ex-situ relocation of rhizomes from endangered habitats is an alternative "method to preserve this genetic diversity, but relocation success is usually unreliable". [102]. Another option is to preserve these species by cultivation. However, this might "be problematic because rhizomes obtained from natural populations tend to" be infected with rot and die after a few years, supposedly "due to viral infection" [100], [102]. However, "this infection appears to be limited to the vegetative parts of the plant" and is not transmitted via seeds. Therefore, the appropriate solution is to produce an ex-situ population by germinating seeds and repeatedly growing new generations [101]. For that, there are need to overcome the seed dormancy and hard seed coat problem, one possible protocol for that is to expose the seeds to scarification and low-temperature stratification [100], [103]. In *Iris lactea* var. *chinensis*. Seed Soaking "in NaOH solution dissolves the seed coat, exposes the endosperm, reduces the mechanical block that prevents the embryo from absorbing water", and possibly removes the physiological "inhibitors produced by the seed" coat; results demonstrate that a combination of NaOH treatment and stratification is an effective practice for breaking *Iris* seed dormancy and improving germination percentage up to 80% [103]. Low temperature stratification also affects the dormancy degree in seeds [104]; this method of scarification and stratification was succeeded in *I. lacteal* Pallas (sect. *Limniris*), and germination percentage increased from 0% to over 80% in treated seeds compared with untreated [103]. A similar protocol was used by Volis *et al*. (2007) "on non-geminating seeds of *I. atrofusca* Baker (sect. *Oncocyclus*)", the results showed a small positive effect on seed germination rates [100]. Some researchers "proposed an in-vitro germination protocol for *Oncocyclus* irises" [33], [105] based on "removing the hard seed coat from unripe fresh seed and then growing the embryo on a growth medium". The most benefits of this method, according to Blumenthal *et al.* (1986) that this method firstly prevents the accumulation of germination inhibitors in the seed coat and also improves the embryo's hygroscopicity required for germination. In the same research, Blumenthal *et al*. (1986) showed that the "high mechanical resistance of the seed coat at the micropylar area is the main cause of seed dormancy". They measured the pressure needed to puncture the seed coat of *Iris lortetii* and *I. atropurpurea*, which "was 135 and 77 atmospheres, respectively" [33]. Another method was used to increase the "germination of Japanese and Siberian iris seeds up to 80-100% by soaking" them in water to remove the germination inhibitors; rainfall and melting snow had the same effect if lasting for a 3-4 month period [106]. Some bearded iris seeds germinate for at least six weeks after chilling below 4.4 °C. Hartmann *et al* (1997) proposed that Abscisic acid level which increases in seeds as they mature, may prevent germination and induce seeds dormancy [107]. *I. rossii* seeds dormancy was broken *in vitro* by storing at 4°C for 30 days before culturing in a "poor medium at 25 °C with the illumination of 3000 lx for 16 h/day" [106]. Various substances might be used to overcome seed dormancy and induce germination. Potassium nitrate (KNO3), thiourea, and gibberellic acid (GA). KNO3 is a widely used substrate for inducing seed germination and can break the dormancy of light-requiring seeds in dark. Thiourea can break the dormancy of both light and chilling-requiring seeds and can also overcome dormancy in seeds with seed coat inhibitors substrates and dormancy due to high temperature [107].

2.4 Current Status Of Iris haynei In Palestine

"Surveying and recording biodiversity can provide valuable information about an area's habitats, plants, animals and natural processes. Information such as the presence (or absence) of species and habitats, the condition of habitats, the size of populations and the type of management in place is useful when planning projects that include or may affect biodiversity" [108]. Regional and local studies have investigated the current status of wild and rare plant species [30], [40], [84], [108]. In global, there are many organizations concerned with recording and assisting in the conservation of rare. Their work is to improve the natural environment locally or globally and inspire everyone to care more about it.

Observation.org (https://observation.org/) is one of the leading nature platforms and a "part of the Observation International Foundation. Volunteers perform a multitude of nature observations worldwide". The deposited data in observation.org "is a powerful tool for nature conservation, research, policy, education and experience". These "data about global biodiversity, past and present, will act as a source of knowledge, facilitate observers around the world through a multilingual global observation system with a species registry for all known species and species groups in nature, flora and fauna, and share a data collection of validated field data through that system with anyone anywhere in the world". The distribution of the genus *Iris* subg. *Iris Iridaceae* and notes on its taxonomy, geographic distribution, and species numbers in Bulgaria were investigated and deposited in observation.org platform [109].

Chapter Three
Materials and Methods

3.1 Field Visits and Specimens Recording

The author investigated the presence of *Iris haynei* in Palestine during a field expedition held between 2020-2022, different localities have been examined in detail with numbers of populations, geographic information, and morphological characters. The findings based on the status of the localities, accenting the targeted species from the investigated genus, sec. *Oncocyclus*. The observed samples of *haynei* deposited in observation.org diversity platform and recorded as well. In adittion, *Iris haynei* were categorized as mature or immature and checked their fructification period.

Figure 4A

Specimens Recording in (faquaa/ south faquaa).

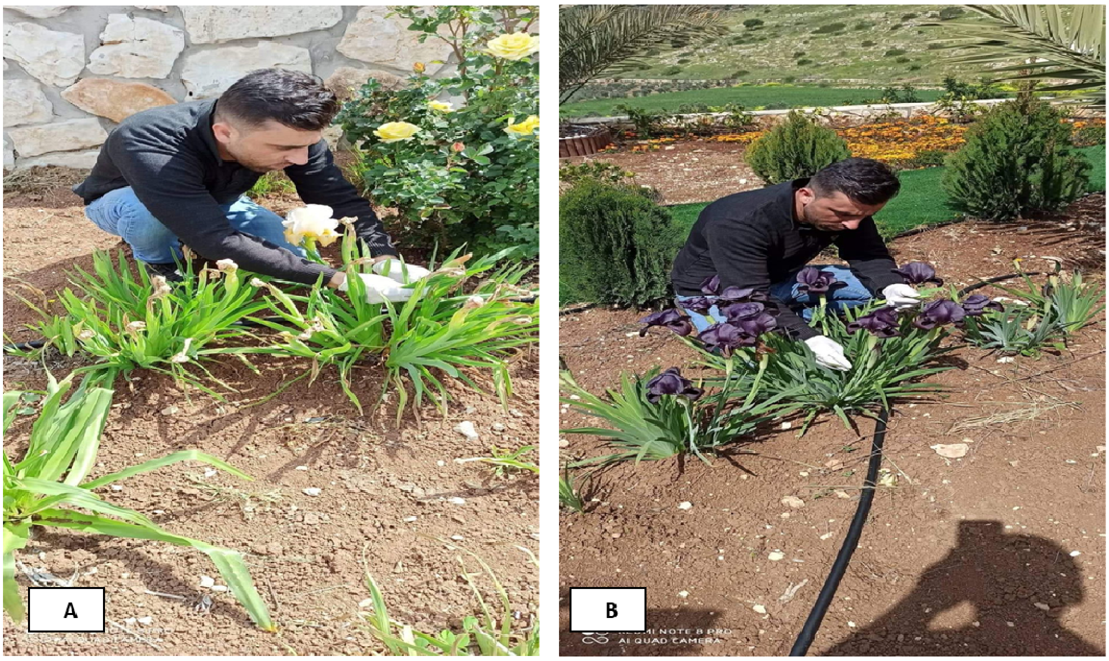
3.2 Sample Collections

When *Iris haynei* showed morphological characterization between February and April, when the flowers were in bloom, at least 20 samples from the leaves next to the flowers were taken for DNA testing.

The samples were randomly selected from scattered places to avoid genetic convergence between the samples. The samples were kept in a fridge at 4 °C degrees for close use [29].

Figure 4 B

Samples collection, (A) Yello (B)purpile for genetic diversity



3.3 Germination Protocol of Iris Seeds

3.3.1 In-vitro trial

A total of 100 Iris seeds are cleaned in tab water and then placed in a flask with distilled water and shaked for three days and were dried on paper towels (figure 5).

Figure 5

seed germination experiment, Iris hanyane seeds

Using a stainless steel hand razor, the seeds are cut 1 mm under the elaiosome under a dissection microscope (Zeiss DV4). The Iris embryo's tip must be visible for the incision to be successful (a small white circle).

To maintain humidity, the sliced seeds are arranged in 10 groups of 10 seeds, with each group being wrapped with wet filter paper.

As an alternative, seeds can be placed in glass Petri dishes that have been lined on both sides with moist cotton or filter paper and covered with parafilm paper.

For two to three weeks, the lots are kept in total darkness at 14 to 15 degrees Celsius. Daily checks are made to ensure that the environment is free of fungus and is safe for the seeds.For the other part of the experiment, another hundred seeds are taken and treated according to the same method in terms of cutting the neck, washing with water and soaking for three days, but they are planted in a medium containing a concentration of 100mg/L of gibberellic acid .

3.3.2 In-vivo trial

Small fields were established in different places, among the places that are considered the home of the lily of Faqoua, and each of these fields has a one hundred seeds, where the seeds were divided into two parts. Part of these seeds was soaked in water for three days and the other part was left as it

3.4 DNA Extraction

Plants have cell walls mostly comprised of cellulose and some other complex polysaccharide or other chemical compounds or have tissues with mucilaginous substances. All these compounds may influence the quality and yield of extracted DNA even in fresh samples and herbarium samples as well. Furthermore, extraction of DNA from herbarium specimens has always been difficult due to the preservation conditions or liquids in which specimens are preserved [85].

For the previus reason young leaves of the *Iris haynei* species had their genomic DNAs extracted using plant DNA mini preparation [85] following the supplier's instructions. Briefly, 50 mg of plant material were added to 500 l of 500 mM NaCl, 100 mM Tris-HCL pH 8.0, 50 mM EDTA, and 10 mM 2-mercaptoethanol, and then the mixture was crushed. After adding 33 Ml of 20% SDS, the slurries were vigorously shaken at 65 °C for 10 minutes. Following the addition of M160 Ml of 5 M potassium acetate, the mixture was centrifuged (Hettich/ Zentrifugen, Germany) for 10 minutes at 4000 rpm. Before being purified using PCIA, the collected supernatant was treated with 2 Ml of RNAase and incubated at 37 °C for 10 minutes (phenol-chloroform-isoamyl-alcohol, 25:24:1). After adding 500 Ml of PCIA to the supernatant, the mixture was centrifuged at 14000 rpm for 10 minutes. The recovered DNA was subsequently precipitated in two liters of cold isopropanol at -20 °C. The pure DNAs were resuspended in 60 l of TE buffer and then kept for later use at -20 °C (10 mM Tris-HCl, pH 8; 1 mM EDTA).

Figure 6

DNA extraction work in the Laboratory, PCR machine in the lab

3.5 Quality measures of the total DNA

DNA quality and concentration were measured with DNA spectrophotometer (Eppendorf A.G., Hamburg, Germany). Another test for DNA quality and concentration was conducted with agarose gel electrophoresis. Samples with poor quality DNA were excluded and the extraction was repeated. Concentration and quality of the used DNA are listed in table 1. For each RAPD reaction, DNA concentration was adjusted at around 200.0 ng/µl.

Table 1

Concentration and quality of the used DNA for RAPD-PCR

Sample no	A260/A280 ratio	Adjusted conc
Yellow	1.9	200 ng/µl
Purpule	1.82	200 ng/µl
Pink	1.67	200 ng/µl

3.6 Primers selections and RAPD-PCR conditions

In order to find the genetic relations between individuals, RAPD primers have been chosen. RAPD can touch many regions in the genome and has the advantage that no prior knowledge of the genome under research is necessary [87]. Ten primers were used in the investigation, which was conducted by Metabion Hy.labs, Ltd. in Israel and (Sigma-Aldrich, USA). In related experiments, the used primers shown excellent polymorphism[29], [110]. Among 10 primers, 6 were selected due to their clarity and variability of fragments.

Table 2

List of RAPD primers for polymorphic DNA generations

Primer Name	Sequence 5`- 3`	References	G/C%
PH-01	AACGCGCAAC	Arif et al., 2010	60%
KFP-6	TCCCGACCTC	Megendi et al., 2010	70%
OPAE-07	GTGTC GTGG	Megendi et al., 2010	60%
OPD-19	CTGGGGACTT	Sudré et al., 2011	60%
OPAG-02	CTGAGGTCCT	Tonk et al., 2010	60%
OPAN-08	AAGGCTGCTG	Tonk et al., 2010	60%
OPB-12	CCTTGACGCA	Tonk et al., 2010	60%
OPJ-06	TCGTTCCGCA	Tonk et al., 2010 Ben el Hadj Ali, et al., 2012	60%
OPG-06	GTGCCTAACC	Ben el Hadj Ali, et al., 2012	60%
OPH-02	TCGGACGTGA	Chowdhury et al., 2002	60%

In order to create polymorphic DNA, 1 (µl) of plant target DNAs were added to a 30 µl PCR mixture along with 1 µl of 10 M primer (sig- ma-Aldrich and metabion hy.labs), Taq quick load 12.5 µl Master mix twice, with a standard buffer. In 1x, there are the following ingredients: 40 mM Tris-HCl, pH 8.9, 44 mM KCl, 3.6 mM MgCl2, 5% glycerol, 0.4 mM each dNTP(A,T,C,G), 25 units/ml of Taq DNA polymerase, 0.06% IGEPAL CA630, 0.05% Tween-20, xylene cyanol FF, and tartraz New England Biolabs is a US company.

The following heat parameters were used with a thermal cycler (Biometra, An Anaylik Jena Company, Germany) to produce RAPD-PCR amplifications: 94 °C for 7 min of preheating, 3 min of initial DNA denaturation, and then 35 cycles of DNA amplification at the following temperatures: 94 °C for one minute, followed by 35 °C and 72 °C for two minutes. After the final extension was finished at 72 °C for 10 min, the amplified products were stored at 4 °C for subsequent gel electrophoresis analysis. The RAPD-PCR products were evaluated in 1.5% (w/v) agarose by gel electrophoresis with 1xTAE (pH 8) running for ??? min, and then were seen under a UV transilluminator and photographed using ethidium bromide (Canon, Japan).

Table 3

PCR thermal cycle used to amplify Ires DNA using RAPD primers

Type of Cycle	Temp. °C	Time , minute	No. of Cycles
Initial denaturation	94	1 min	1
Denaturation	94	3 min	
Annealing	35	2 min	35x
Extention	72	2 min	
Final Extention	72	10 min	1

Chapter Four
Results

4.1 Morphology and description

I. haynei, often known as the Faqqua Iris, has smooth, lance-shaped, greyish-green leaves. Long, thin stems with enormous, fragrant blooms in colors of deep purple, brownish purple, or dusky lilac in the spring (between March and April). In temperate areas, it is rarely grown as an attractive plant since it requires extremely dry summer conditions. A geophyte 30-60 cm tall grows in clumps depending on the quantity of rain in a given year. The rhizome is thick and lacks stolons. The leaves are sword-shaped, up to 40 cm long, 8-15 mm wide and arranged in fan-like, 5-8 leaved clusters. The flower is single, spectacular and fragrant, 10-12 cm across, usually longer than its width (Figure 7A).

4.2 Field surveys and localization of *Iris haynei* in Faqqua

90% of the Iris flower's population in Palestine is centered in the Faqqua highlands, were have a consecrated distribution in the northern and southern mountains of Faqqu'ah and west of the town of Faquaa, extending from the north between Faquaa and Jalboun. From the northern side, it is found among farmers' fields and olive trees, all the way to the plains of Arbouna, in addition to its presence in the gardens of homes where they are taken care of, while the remaining 10% is scattered throughout the Tubas Governorate in places like Yarzeh, Yasid, the Talfeet Mountains, and the Al-Mughayer Mountains.

In Faqqua Mountains, its distribution is centered on the south side, where it germinates to a degree of around 50%. The flower is socially organized into groups that can contain up to 200 blossoms (Except two areas where locals make special gardens, Sawsan Faqqua garden, located in the center of the old town, has about 2000 flowers, and the other garden for Mr. Mounir Salah, whose flowers are in various colors, especially yellow and purple, and its counts around 1000 flowers).

The remaining flowers were dispersed individually or in groups throughout the Faqqua Mountains because sociable flower plants are typically found living in groups. In the area of the Faqqua mountains, there are about 6000 individual flowers. The observed samples of hanyane deposited in observation.org diversity platform (https://observation.org/).

Figure 7 .A

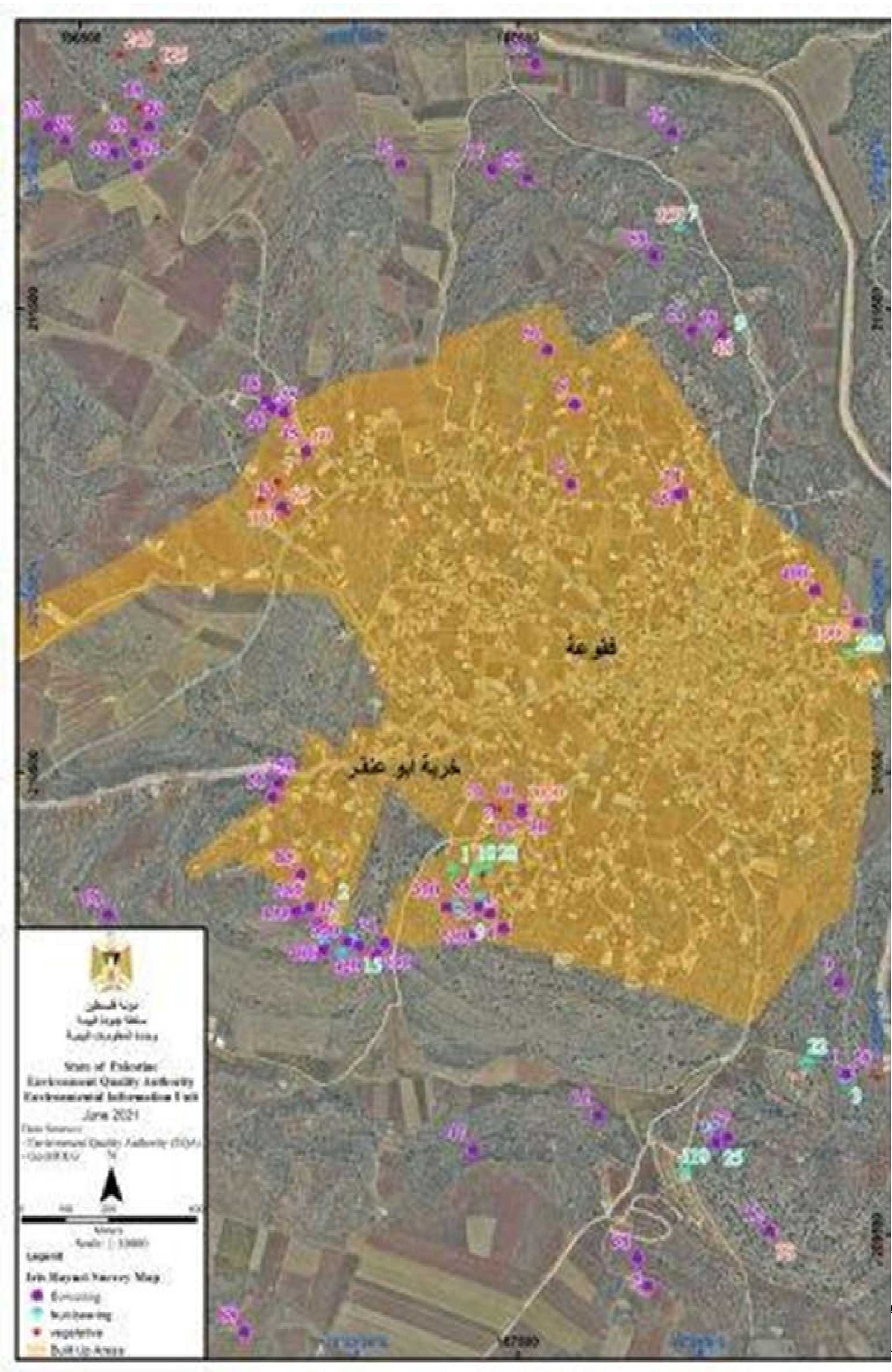
Iris haynei or Faqqua Iris on their habitat

Figure 7 . B1

landscape view of Faqqua village

Figure 7. B2

localization of Iris haynei in Faqqua



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or left in the moist filter paper for further development. The seeds were examined twice daily during this phase of the experiment to make sure that no mold was forming.

The seeds were moved into a growth chamber with a base temperature of 19–20 oC, base humidity of 70%, and a 12h day/12h night light cycle once the new developing root was 2 mm long.

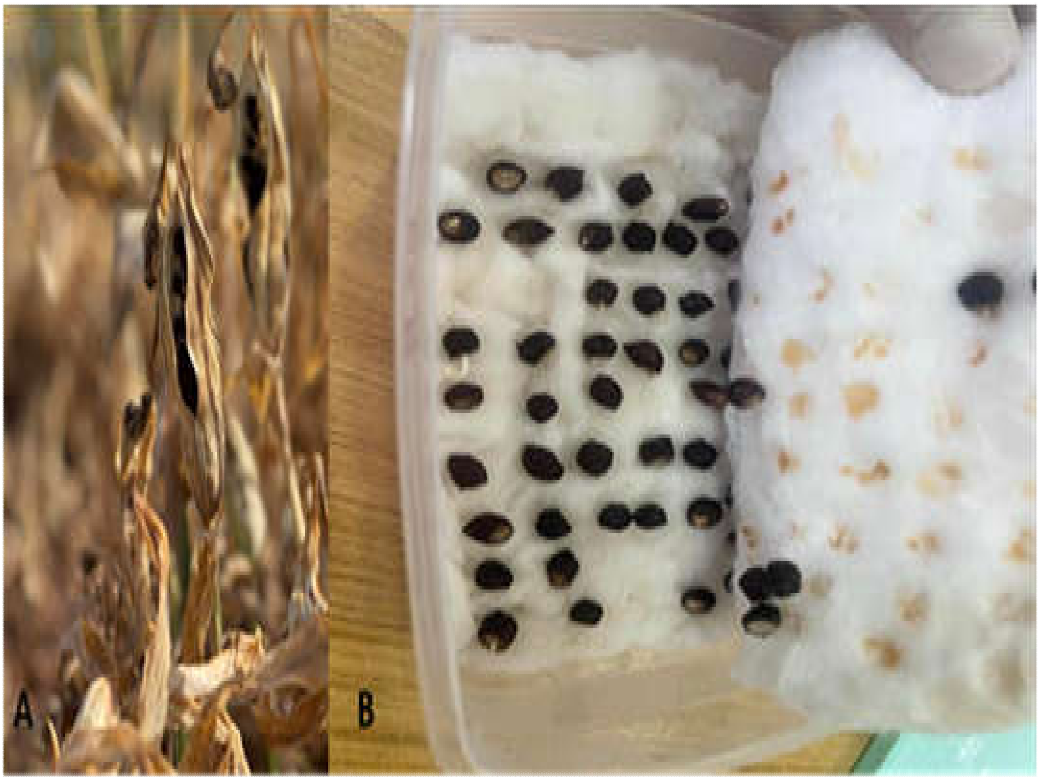
Pure presoaked perlite with a water absorption rate of 60% was employed as the growing medium in the growth chamber. Every day, regular tap water was used to lightly sprinkle the perlite.

The seedlings' strong root development in just two weeks resulted in exposed root mass in the atmosphere. The seedlings needed to be moved at this point, or the roots needed to have moistened perlite placed over them.

The leaves first appeared eight days later. This suggests that it is necessary to move them into separate sterile 8/9 cm pots, fill them with pure perlite that has been presoaked in soil, and set them up to continue growing in the growth chamber.

Figure 8

Iris haynei seeds



(A) Collected seeds from the mother plant. (B) Seed germination in the laboratory.

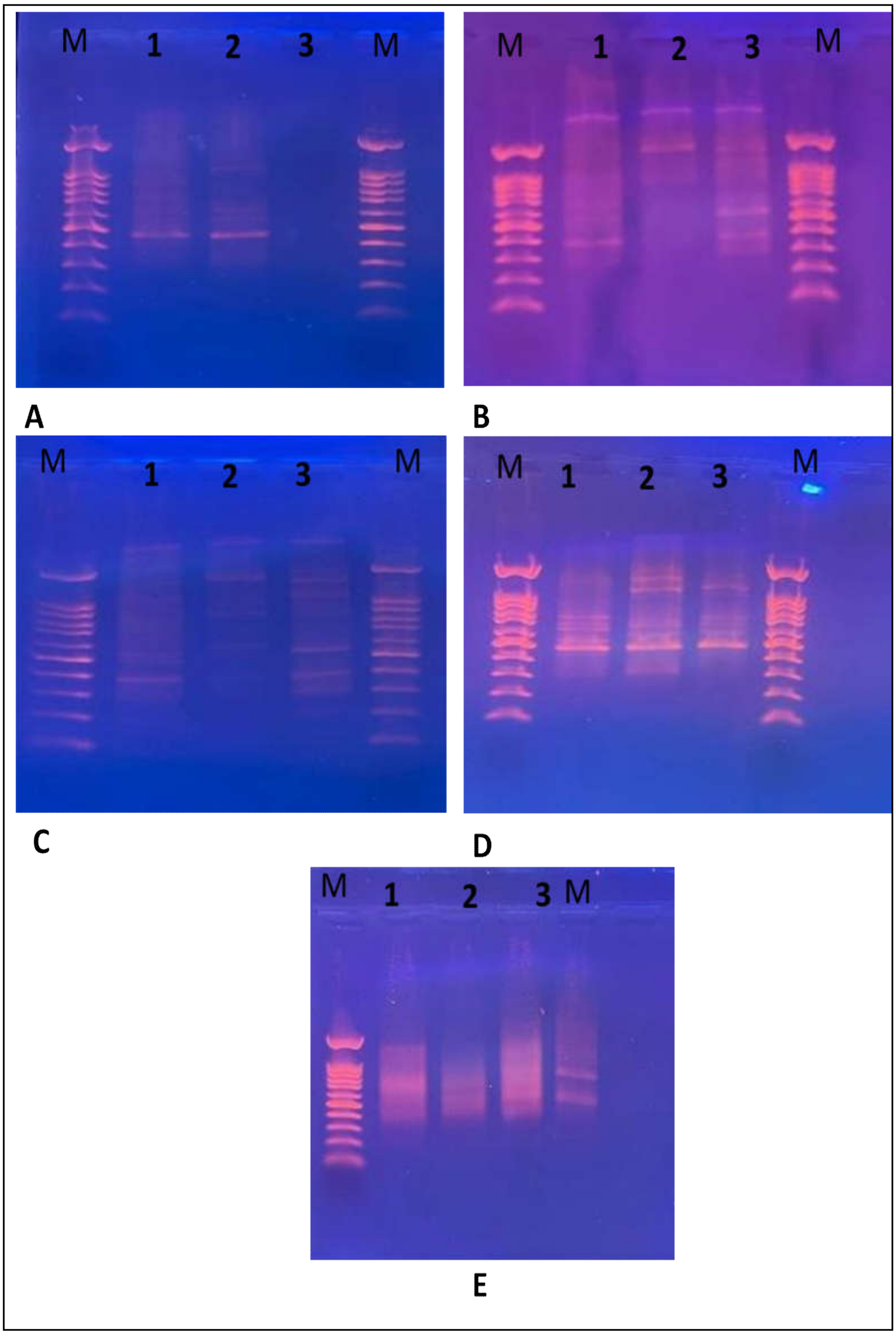
4.4 Genetic diversity

Depending on whether there are amplified bands or not,DNA polymorphisms generated by RAPD-PCR were evaluated as present (1) or absent (0) in all three *Iris haynei* species. The only bands with a distinct major key were scored. A primer number and an estimated base pair size for the amplified fragment were used to determine the individual bands that were helpful for distinguishing species. A dendrogram illustrating the genetic relatedness of Iris species was generated using genetic distance computed based on the Jaccard coefficient after doing a pairwise comparison between them using the proportion of common bands obtained by each utilized primer (20) using the correlate module of SPSS NTSYSpc version 19. A common estimate of genetic identity, the Jaccard's coefficients were calculated using the number of bands shared by the samples, the amplified fragments in sample A, and the fragments in sample B. Based on these indexes, similarity matrices were generated. This was done using SPSS NTSYSpc version 19's categorize module.

Development of Polymorphic DNA: Using RAPD, the isolated DNAs from various iris species were examined for the development of polymorphic DNA fragments. It was discovered that these primers (PH-01, KFP-6, OPAE-07, OPD-19, OPAG-02, OPAN-08, OPB-12, OPJ-06, OPG-06, OPH-02) create fragments for the analyzed plant species independently, proving their dependability in addition to their sensitivity and specificity (Table 1). The For each analyzed species, the RAPD banding patterns are consistent and repeatable. were confirmed by conducting two tests on these primers. Amplicons from 50 to 1500 bp were produced by the RAPD profiles when all samples were used at once. The three Iris species that were chosen were related to one another genetically using the polymorphism produced by the primers (OPAN - 08, OPAG - 02, OPB - 12, OPG - 06, and OPAG2).

Figure 9

Gel views for DNA of Iris samples amplified by using different RAPD primers, and their patterns



(A) OPAN -08; (B) OPG - 06 ; (C) OPB – 12; (D) OPAG2 and (E) OPAG – 02 electrophoretically evaluated on 1.5% agarose gel. (M) stood for PCR Marker. [100-1500 bp]; while the numbers (1) to (3) referred to Iris haynei species color: yellow, purple and pink respectively.

The polymorphism generated by the used primers (OPAN – 08; OPAG – 02; OPB – 12; OPG – 06; OPAG2) respectively. When there is a band (1) and when there is no band (0), (Table 2).

The phylogenetic trees of the Iris three samples were constructed using SPSS NTSYSpc (Figure 10 A). Genetic data analyzer NTSYSps V. 2.10e NTSYSpc was used to fined the similarity values between species as shown in (Figure 10 B).

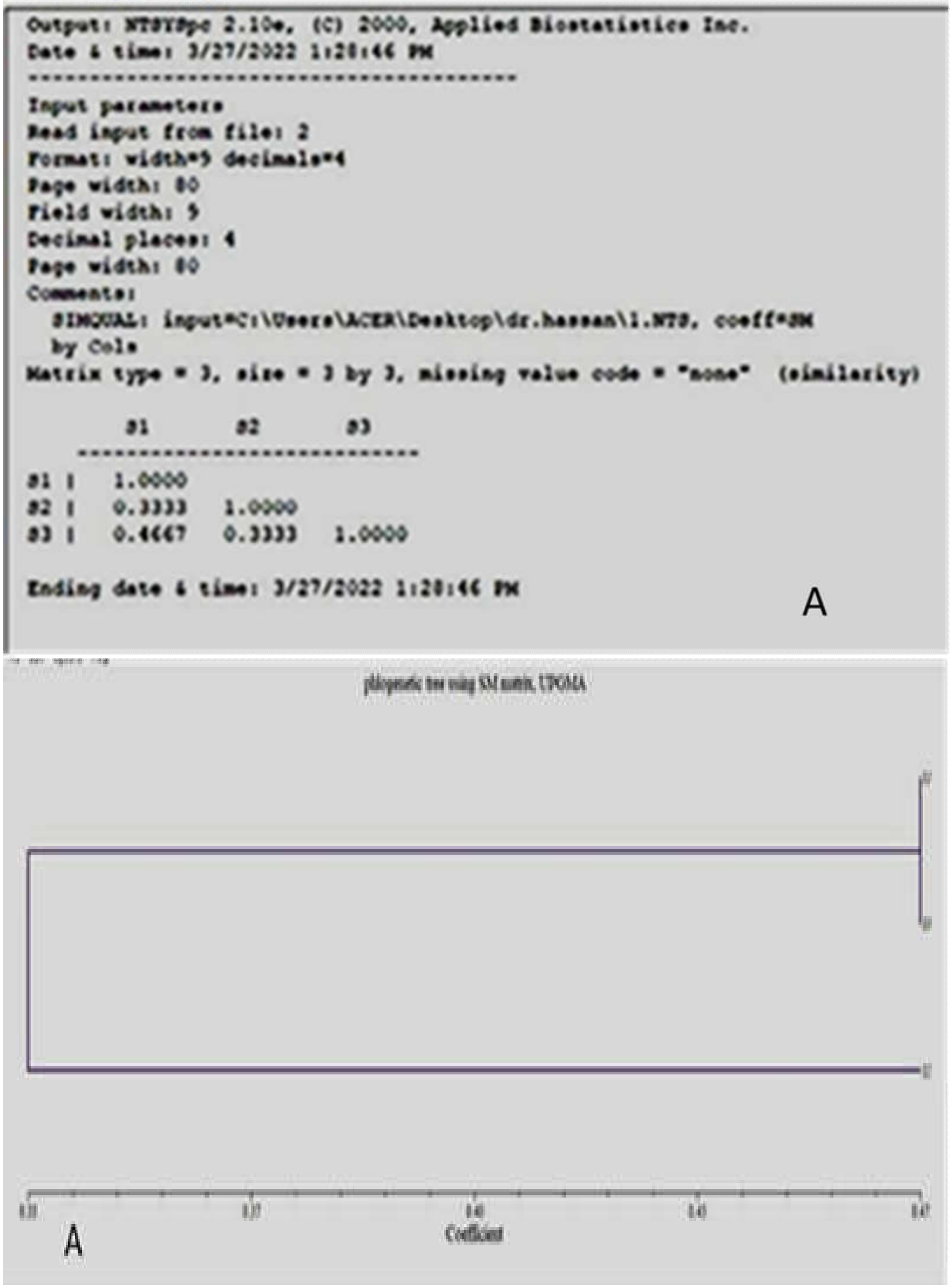
Table 4

RAPD primer polymoriphism, (Monomorphic and Polymorphic bands)

Primers	Total No. of bands	No. of Monomorphic	No. Polymorphic	Polymorphic %	Monomorphic %
OPAN	6	0	6	100%	0%
OPAG-02	7	2	5	71,5%	28.5%
OPB-12	6	0	6	100%	0%
OPG-06	10	0	10	100%	0%
OPAG-2	8	3	5	62,5%	37,5%

Figure 10.A

Similarity matrix between species, Similarity & Dissimilarity of three Irises samples involved in the genetic study



```
Output: NTSYSpc 2.10e, (C) 2000, Applied Biostatistics Inc.
Date & time: 3/27/2022 1:28:46 PM
----------------------------------------
Input parameters
Read input from file: 2
Format: width=5 decimals=4
Page width: 80
Field width: 5
Decimal places: 4
Page width: 80
Comments:
  SIMQUAL: input=C:\Users\ACER\Desktop\dr.hassan\1.NTS, coeff=SM
  by Cols
Matrix type = 3, size = 3 by 3, missing value code = "none"  (similarity)

        S1       S2       S3
    ----------------------------
S1 |   1.0000
S2 |   0.3333   1.0000
S3 |   0.4667   0.3333   1.0000

Ending date & time: 3/27/2022 1:28:46 PM
```

Figure 10 .B

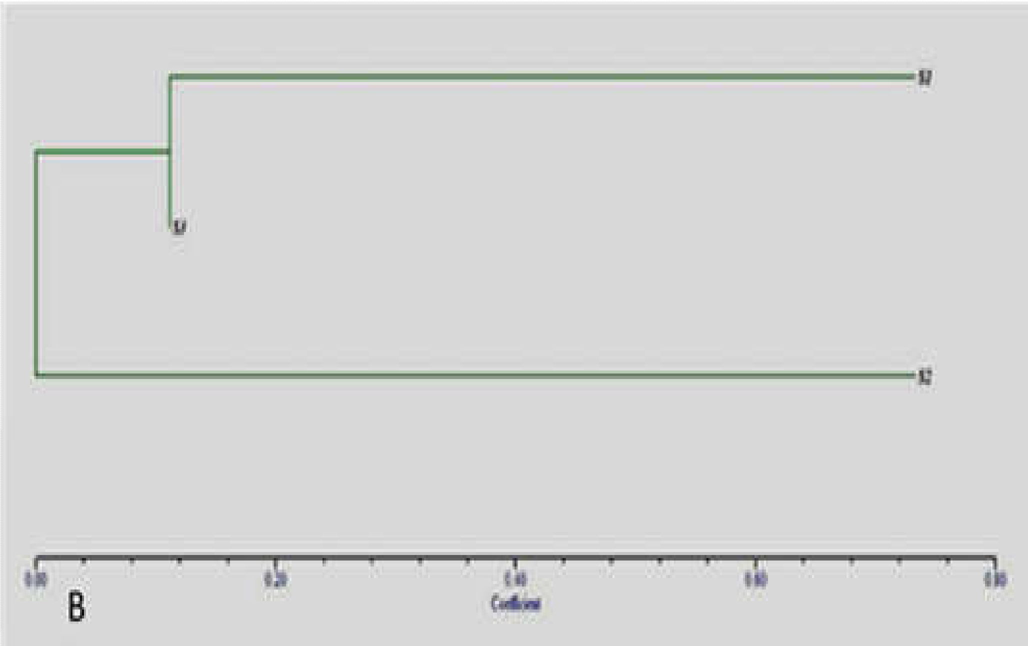
The UPGMA dendrogram cluster tree was generated by using a classified module of SPSS NTSYSpc, for three Irises haynei species

```
Output: NTSYSpc 2.10e, (C) 2000, Applied Biostatistics Inc.
Date & time: 3/27/2022 1:41:22 PM
----------------------------------------
Input parameters
Read input from file: 4
Format: width=5 decimals=4
Page width: 80
Field width: 5
Decimal places: 4
Page width: 80
Comments:
  SIMGEND: input=C:\Users\ACER\Desktop\dr.hassan\1.NTS, coeff=NEI72, dir=Cols
Matrix type = 2, size = 3 by 3, missing value code = "none"  (dissimilarity)

         S1        S2        S3
    ----------------------------
S1 |   0.0000
S2 |   1.4663    0.0000
S3 |   0.5476    0.7708    0.0000

Ending date & time: 3/27/2022 1:41:22 PM
```

B



S1=yellow, S2 pink =,and S3= purpile

Using SPSS NTSYSpc, similarity coefficients with the Jaccard measure were generated using data from RAPD profiles scanned from the three species of Irises using five reproducible primers. For the primers, The evaluated plant species' banding patterns have pairwise similarity ranging from 0.33 to 0.47. (OPAN – 08; OPAG – 02; OPB –

12; OPG – 06 and OPAG2). Yellow and purple *Iris haynei* showed the highest pairwise similarity scores (0.47), indicating that they are the most closely related species. Pink and yellow *Iris haynei* had the lowest similarity score, (0.33), making them the most genetically diverse.

4.5 Discussion

Palestine's environment places a special emphasis on species of *Iris haynei*. The Palestinian government views it as a national plant despite the fact that it poses a minimal threat [111]. Generally speaking, they can be found in ranges ranging from Marj Ibn Amer to the Bisan and Faqqua mountains. Irises from the Faqqua Mountains flourish in the unique climate there, where they grow in a region known for its natural diversity and picturesque landscape. The *Iris haynei* species in Palestine has been the subject of numerous investigations and projects. In order to preserve it, the Environmental Quality Authority surveys this bloom on-site in collaboration with the Wildlife Society in Palestine. This was done as part of a national initiative to safeguard Palestine's national flowers [111].

The findings from seed germination trials include the following: In a lab setting, 50% of seeds germinated in the presence of the hormone gibberellic acid, while 34% did not. This demonstrates the hormone gibberellic acid's beneficial impact on seed germination.

There were two experiments conducted. In both tests, 100 seeds were placed in water for three days to preserve them, while the remaining 100 seeds were planted immediately. None of the seeds had sprouted in the first year of the two studies. In both tests, 25% of the seeds have developed by the second year. On the other hand, the Iris genus seed requires 5–7 years to fully mature, as noted in the literature reviews.

Two tests have been carried out in both the field and the lab. In both tests, none of the seeds have developed after the first year. In both tests, 25% of the seeds have developed by the second year. It follows that a deeper cut will more effectively reach the tip of the iris radicle without harming the meristem and will lead to a higher level of germination percentage. The depth of seed cuts also plays a significant impact in growth percentage.

The Iris genus seed, on the other hand, requires 5-7 years to fully mature, according to the literature evaluations. Experimental seed germination has many difficulties, such as defective seeds, bird feeding, farm activities burying the seeds, and plant growth.

Ex-situ conservation and in-situ conservation are two notions that underpin conservation efforts. The Laboratory for Seed Germination and Conservation, which functions similarly to the seed bank at the National Agriculture Research Center in Jenin, is where ex-situ techniques are most frequently used. Here, seeds are gathered from all over Palestine and kept in low humidity and temperature conditions. Ex-situ methods are also used in the botanical labs of numerous research facilities around the West bank.

The in-situ strategy involves the recovery of healthy populations of species as well as the conservation of natural habitats, ecosystems, and the surrounding areas where they have established distinctive traits. All of these decisions must be made on-site using mechanisms for designating, managing, and monitoring. This entails the creation of designated protected areas as well as local species protection efforts. In terms of preserving wild species, this kind of conservation is a fairly effective strategy [112].

The ex-situ strategy, on the other hand, entails the preservation of biological diversity components outside of their natural habitat. Methods for sampling, transferring, and storing data are included. This kind of care is regarded as static and concentrates on seed, DNA, pollen, and botanical gardens storage. Although less effective than in-situ conservation, this technique is frequently employed to build databases and safe genetic resource storage facilities for wild species [112]."Quasi-in-situ" conservation is the complementing of both ex-situ and in-situ techniques with a "Reintroduction" phase. This method connects ex-situ and in-situ conservation efforts and is essential for improving the life cycle of the target species. [113].

Assessing the genetic and morphological variability of the planted Iris was one of the study's main goals. Additionally, assess the region of Faqqua-Iris Jenin's haynei diversity's current state. The simplest molecular markers, RAPD, were used to identify and study three different iris species in order to determine their genetic links with one another. Out of eleven universal primers, five (OPAN-08, OPAG-02, OPB-12, OPG-06, and OPAG2) were found to produce a disproportionately high level of DNA polymorphism among the three species of Iris.

The results of this study might suggest that the chosen primers employed in RAPD testing were capable of producing polymorphic bands on the most genetically diverse loci in the Irises species, as shown by [114].

According to earlier RAPD research studies, the primers OPD-19, OPH-02, and OPAN-08 were able to generate 7, 6, and 2 bands, respectively. For instance, when employed to analyze the genetic diversity of cultivated and wild Moringa olefera Lam, the KFP-6 primer yields 6 bands. OPD- 19 primer yielded 2 bands when utilized in the molecular characterisation of 27 wild hawthorn genotypes, while provenances and evaluated by RAPD markers [110]. In the genetic variability study of selected Turkish oregano (Origanum onites L.) clones, these primers, OPAG-18, OPAG-06, OPAC-12, OPAG-02, and OPG-07, were able to generate 3, 5, 5, 6, and 6 polymorphic bands using the RAPD Technique, indicating that small numbers of polymorphic bands were typical for that.

The same outcome was discovered in a second RAPD investigation of Thymus species growing in Turkey's eastern Anatolia area using primers OPA-2, OPH-16, and OPH-18. A total of 8 polymorphic bands were created [115]. When compared to other studies, the similarity score achieved utilizing the five primers in this research study was sufficient to identify the genetic links among the chosen Irises species submitted to RAPD tests.

Sequence-based analysis was unable to discriminate between the several species of thymes in prior research because of the striking similarity between their DNA sequences in the amplified region. So far, the three different iris species that were the topic of this study have been distinguished unambiguously by RAPD primers (OPAN - 08; OPAG - 02; OPB - 12; OPG - 06 and OPAG2). Additionally, as RAPD analysis reflects both coding and non-coding sections of the genome, RAPD primers were able to differentiate taxa below the species level [113]. Therefore, it should not come as a surprise that RAPD, employing the same approach with the same or different primers that were employed in these research, was able to detect the genetic diversity among several plant species in Palestinian figs, Faba beans, wheat,...etc. The three distinct

Irises species could be distinguished in this study using RAPD technique. For the yellow, purple, and pink Iris, it was found that the OPAN primer produced 3, 1, and 5 distinct bands, respectively. For the yellow, purple, and pink Iris, the primer OPAG-02 produced 4, 5, and 0 distinct bands, respectively. The study also discovered that the primer OPG-06 distinguishes between the Irises species by providing 3, 2, and 8 distinct bands for yellow, purple, and pink Iris, respectively; 3 of these bands shared characteristics with purple and pink Iris. For the yellow, purple, and pink Iris, the primer OPB-12 produced 2, 3, and 5 distinct bands, respectively. Additionally, for the yellow, purple, and pink Iris, the final primer OPAG2 produced 5, 6, and 7 distinct bands, respectively.

The researchers suggested using these results to specifically recognize these species or extending that study to additional medicinal plants because they were thought to be promising for future DNA fingerprinting investigations on thyme species variants. The primers OPAE-09, which only created a single polymorphic band (26), and OPC-6, which only produced a single specific band to Tinospora cordifolia, Emblica officinalis, and Tribulus terestris, respectively, were used in these research on genetic divergence among Dimorphandra spp. accessions, were used to confirm this discovery [116]. This study successfully revealed the genetic diversity of a few chosen *Iris haynei* species that could be extremely valuable for any hypothetical Irises breeding program that could be carried out in Palestine. Any plant breeding effort will succeed more if its breeding genotypes are diverse and genetically linked. The different Irises haynei species selected for this study could all be distinguished clearly using the RAPD procedure, and it could also be used to distinguish Irises species from any mixed populations. Many other researchers had successfully used a similar approach for molecular diagnosis of various species and cultivars [117]. Additionally, since the RAPD methodology had been utilized to identify the various components contained in herbal formulations, these markers might be employed as the preferred method for identifying components for complicated herbal medicines (30). These will therefore have a big impact on quality control. In addition to providing details on genomic diversity below the species level [90], RAPD also yields results very quickly and costs less money [118]. It is important to note that the RAPD bands pattern found in this study may be duplicated in our lab and experimental settings. To overcome the disparities left unsolved by RAPDs, more research for finer molecular analysis of medicinal plant genotyping were advised. Three iris species growing in Palestine could be distinguished thanks to the application of RAPD markers. These markers can be successfully employed for clonal selection and cultivar improvement in the future. These findings can also be utilized to determine the ideal conditions for iris seed germination and to modify the genetic factors affecting horticulturally significant features. Aside from other molecular markers like AFLP, ISSR, and SSRs, RAPD markers have been shown to be beneficial for thyme germplasm characterisation and diversity study. Therefore, our findings offer recommendations for identifying Thyme species, as well as support for their future sustainable utilization to mitigate stressors from both natural and human sources on these priceless natural resources.

Given this taxonomic confusion, along with the high variation observed in natural populations and transitional populations frequently encountered in the field, more detailed research is required on patterns of morphological variation.

Plant conservation efforts have been based mainly on estimations of vulnerability, which may vary among countries and conservation organizations. The World Conservation Union (IUCN) has developed five quantitative criteria of vulnerability for evaluating the extinction probability of species [119], [120]. (EX)for Extinct, the term is used when there is no reasonable doubt that the last individual of the species has died), (CR) which meaning that more than 75% reduction and the term used to refers to Critically Endangered (in grave danger of extinction, considered as a species facing an especially high risk of extinction in nature), Endangered (EN=50-75% reduction) (in danger of extinction, considered as a species facing a very high risk of extinction in nature), Vulnerable (VU= >30-50% reduction), the term is use to discripe the species that facing a high risk of extinction in nature), and Near Threatened (NT) (expected to be endangered soon).

These categories have been widely accepted worldwide and form the basis for the IUCN Red List of Threatened Plants [119]. The IUCN criteria consist of a set of decision rules based mainly on

quantitative thresholds of population size, distribution range, rate of declining and extinction probability [119], [120]. These criteria are considered the first step in conservation, expressing the estimation of extinction probability. The next step should be setting priorities for the species for conservation. A priority-setting process was suggested by Coates and Atkins [121]. Such a process was based on the risk of extinction at the population, taxon and ecological community levels, genetic structure and population ecology. The method requires extensive surveys to generate the necessary data needed. Setting an urgent conservation policy is necessary for most countries where such data are scarce or unavailable.

The locational information, according to the field investigations conducted in this study was relevant to similar literature, the existing herbarium collections from the Palestinian territory were shown and the representativeness of the collections is summarized in Table X

similar works worldwide to situate the Irises sec. Oly, where some species were found to be endemic in their habitats, at the same line the five species of Royal irises were endemic in the Palestinian habitat as well as other species of Irises are native to other habitats but they are very similar to Palestinian Irises Dimopolous et al., 2018-2019).Adamović, 1901.

Today, the vast majority of the described binomials of Iris sect. *Oncocyclus* is widely distributed throughout the Fertile Crescent of southwestern Asia [39], [42]. Although little is known about the phylogenetic relationships and historical biogeography of these taxa, there is still reason to believe that populations of haynei in palestine have colonized their actual distribution range. Zohary *et al* (1973) proposed that southwest Asian steppe forest species could have expanded into the Near East during the hot and dry interpluvial cycles of the Pleistocene [42]. Among these invading species, Zohary (1973) also included the Irano-Turanian elements Pistacia and Retama, both of which are closely associated with Iris haynei. Moreover, during the Holocene, when human activities of perturbation increased in the Near East, this pattern of Irano-Turanian immigration may have intensified [122]. Consistent with such a scenario of unidirectional range expansion, one of the upgma phenograms shows a striking pattern of sequential relationships among populations from north to south. A direct interpretation would be that the northern populations (haynei) are likely the source populations from which the central/southern populations descended. However, in previously studies , the genetic diversity analysis provides no evidence that these populations passed through severe bottlenecks because high levels of diversity were found in nearly all the populations studied.

This study is investigating one of the symbolic flowering and endemic listed as threatened plants in the Palestinian habitat (Iris haynei). It is called "royal iris" and it's limited to the northeast of the West Bank, particularly the village of Faqqua. A better understanding of the current status of the Iris haynei population to ensure their continuity in the Palestinian ecosystems.

The study was implemented by conducting field surveys to localize every plant on site and to check the fructification period (in-situ techniques). Past transplantation was monitored on-site and manual pollination was conducted and tested. On the other hand, seeds of the selected species were collected for the seed bank to develop germination protocols (ex-situ techniques). Finally, after the lab studies have been conducted, the plants generated are ready to reinforce their original populations in their natural habitat (in-situ techniques). Besides, this study investigated the morphological and genetic variations of the planted Iris. For that, several Iris haynei species were grown in Faqqua Palestine and experiments were done, including seed germination, genetic variation and site environmental scanning. In lab seed germination, 50% of seeds have been germinated in the presence of Gibberellic acid, while without hormone 34%. In the first year, none of the seeds have grown in the field, while in the second year, 25% have grown. In" this study, the RAPD method was used for genotype identification and characterization of "three Iris haynei colors (yellow, purple and pink). Five out of ten primers were tested for their ability to generate polymorphism from selected Iris haynei using RAPD-PCR, and the obtained data were analyzed. The similarity value analysis was 0.46 between (yellow and purple) Irises, while the similarity value between (yellow and pink) was 0.33. In this study, genetic diversities of studied were Iris haynei colored species ascertained successfully using RAPD markers, concluding that it could be a useful tool for identifying Iris species.

List of Abbreviations

Abbreviations	Meaning
EQA	Enviromental Quality Authority
SPSS	Statistical Package for Social Sciences
RAPD	Amplified Polymorphic DNA
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
AFLP	Amplified Fragment Length Polymorphism
RFLP	Randomly amplified polymorphic DNA
EDTA	Ethylenediamine tetraacetic acid
SDS	Sodium Dodecyl sulfate
RPM	Round Per Minit
TAE	Tris-Acetate EDTA
DNA	Deoxy ribonucleic acid
RNase	Ribonuclease
TBE	Tris/Borate/EDTA
UV	Ultraviolet
PCIA	phenol-chloroform-isoamyl-alcohol
IUCN	International Union for conservation of Nature

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جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

الاستقصاء البيولوجي والوراثي للحفاظ على السوسنة الفلسطينية في فقوعه

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم البيئية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

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الملخص

خلفية الدراسة: سوسن فقوعة من النباتات المزهرة التي تعيش في الشمال الشرقي من الضفة الغربية وتعتبر رمز لدولة فلسطين.

الهدف الهدف الرئيسي من هذه الدراسة هو خلق فهم أفضل للوضع الحالي لسوسن فقوعة، وذلك لضمان استمراريتها في النظم البيئية الفلسطينية.

الطريقه والإجراءات: تم إجراء مسوحات ميدانية لبعض الأنواع لتحديد موقع كل نبات في الموقع والتحقق من فترة الإثمار(تقنيات في الموقع). تمت مراقبة عمليات الزرع السابقة في الموقع وتم إجراء التلقيح اليدوي واختباره. من ناحية أخرى، تم جمع بذور الأنواع المختارة لبنك البذور لتطوير بروتوكولات الإنبات (تقنيات خارج الموقع). أخيرًا ، بعد إجراء الدراسات المخبرية، تكون النباتات التي تم إنتاجها جاهزة لتعزيز مجموعاتها الأصلية في بيئتها الطبيعية (تقنيات في الموقع).

إلى جانب ذلك بحثت الدراسة في الاختلافات الشكلية والوراثية للسوسن. لذلك تم زراعة العديد من أنواع السوسن في بلدة فقوعة وأجريت تجارب منها إنبات البذور والتنوع الجيني والمسح البيئي للموقع.

النتائج: في تجربة إنبات بذور المختبر، تم إنبات 50٪ من البذور في وجود الهرمون النباتي حمض الجبريليك بينما بدون هرمون 34٪. بينما في الحقل في السنة الأولى، لم ينمو أي من البذور وفي السنة

الثانية نمت 25 ٪ من البذور. في هذه الدراسة، تم استخدام طريقة RAPD لتحديد الأنماط الجينية وتوصيف ثلاثة ألوان من سوسن فقوعة هي (الأصفر والبنفسجي والزهري).

خمسة من أصل أحد عشر برايمرات تم اختبار قدرتها على توليد تعدد الأشكال من سوسن فقوعة باستخدام RAPD-PCR وتم تحليل البيانات التي تم الحصول عليها حيث كشف تحليل التشابه الزوجي عن قيمة التشابه بين اللونين الأصفر والأرجواني وكانت 0.46 بينما قيمة التشابه بين اللونين الأصفر والوردي 0.33. من خلال هذه الدراسة، كانت التنوعات الجينية التي تمت دراستها هي الأنواع الملونة وتم التحقق من نجاح هذا الطريقة. وخلصت الدراسة إلى أنه يمكن أن تكون أدوات مفيدة لتحديد أنواع السوسن.

الكلمات المفتاحية: سوسنة فقوعه، الزهره الوطنيه، النظام البيئي.