T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

MORPHOLOGICAL, PHYSIOLOGICAL, AND MOLECULAR SCREENING OF WILD FLOWER SPECIES OF ALADAG MOUNTAINS WITH SPECIAL PERCEPTION ON LANDSCAPE HORTICULTURE

MEHTAP VURAL

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Doctor of Philosophy Thesis

Supervisor

Prof. Dr. Sedat SERÇE

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The study titled **"Morphological, Physiological, and Molecular Screening of Wild Flower Species of Aladag Mountains with Special Perception on Landscape Horticulture"** is presented by **Mehtap VURAL** under the supervision of Prof. Dr. Sedat SERÇE and accepted as a Doctoral thesis by the jury at the Department of Agricultural Genetic Engineering of the Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences.

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This thesis has been found appropriate at the date of ..../..../2021 by the jury mentioned above who have been designated by Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated …./…./2021 and numbered …… **…/…/2021**

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THESIS DECLARATION

I hereby declare that thesis was written by myself. Every detail in this thesis document was gathered, organized and presented in accordance with the scientific and academic guiding principles as well as the thesis writing regulations of the institute of natural and applied sciences, Niğde Ömer Halisdemir University. All information sourced from other researchers have been duly referenced.

Mehtap VURAL

SUMMARY

MORPHOLOGICAL, PHYSIOLOGICAL, AND MOLECULAR SCREENING OF WILD FLOWER SPECIES OF ALADAG MOUNTAINS WITH SPECIAL PERCEPTION ON LANDSCAPE HORTICULTURE

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Globally ornamental plants are grown due to their aesthetic value. These plants offer beauty and attractiveness to living spaces, as well as providing economic value. It is therefore important to know the reproduction and cultivation characteristics of annual and perennial plants that can be used as cut flowers, dried flowers, ground covers, indoor and outdoor plants. For this purpose, *Consolida orientalis* and *Consolida raveyi* were used in this study. Seed germination and dormancy are the main factors for seedling emergence and robust growth of plant throughout its life cycle. Some plants require some pre-sowing treatment before germination and the plants for this study fall in this category. Because of this characteristic, cold stratification was performed for plants in this study. Plant growth regulators were used to prime seeds to ascertain their impact on seed germination. Morphological characteristics were also measured for these plants grown under greenhouse condition. In addition, the iPBS marker system was used to estimate plant taxon closely related to the plant species used in this study.

Keywords: *Consolida orientalis*, *Consolida raveyi*, landscape, seed germination, cold stratification, seed priming, morphology, molecular characterization

ÖZET

ALADAĞLARDAKİ YABANİ ÇİÇEK TÜRLERİNİN PEYZAJ BİTKİSİ OLARAK KULLANIMLARININ DEĞERLENDİRİLMESİ ÜZERİNE MORFOLOJİK, FİZYOLOJİK VE MOLEKÜLER TARAMASI

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Süs bitkileri estetik ve görsel özellikleri nedeniyle tüm dünyada yetiştirilmektedir. Bu bitkiler yaşam alanlarına güzellik ve etkileyicilik kazandırmanın yanı sıra ekonomik olarak kazanımlar da sağlamaktadır. Bu nedenle kesme çiçek, kurutulmuş çiçek, yer örtücü, iç ve dış mekan bitkisi olarak kullanılabilecek tek yıllık ve çok yıllık bitkilerin çoğalma ve yetiştirme özelliklerinin bilinmesi oldukça önemlidir. Bu amaçla, *Consolida orientalis* ve *Consolida raveyi* bitkileri tercih edilmiştir. Tohum çimlenmesi ve dormansi, fide çıkışı ve bitkinin yaşam döngüsünü etkileyen faktörlerdir. Bazı bitkiler çimlenmeden önce soğuk işleme ihtiyaç duymaktadırlar. Bu nedenle, bu çalışma kapsamında soğuk katlama uygulaması yapılmıştır. Bitki büyüme düzenleyicilerinin tohum çimlenmesi üzerindeki etkilerini tespit etmek amacıyla priming uygulaması yapılmıştır. Sera koşullarında yetiştirilen bitkilerin morfolojik özelliklerine ait ölçümler alınmıştır. Ayrıca, çalışma kapsamında kullanılan bitki türlerine yakın akraba taksonları tespit etme amacıyla iPBS markör sistemi kullanılmıştır.

Anahtar Sözcükler: *Consolida orientalis*, *Consolida raveyi*, peyzaj, çimlenme, soğuk katlama, tohum priming, morfoloji, moleküler karakterizasyon

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SYMBOLS AND ABBREVIATIONS

Symbols	Descriptions
%	Percentage
°C	Degree celcius
µL	Microliter
cm	Centimeter
g	Gram
h	Hour
L	Liter
M	Molar
mL	Milliliter
mm	millimeter
mM	Millimolar
ppm	Parts per million
rpm	Revolutions per minute

Abbreviations	Descriptions
2iP	Isopentenyl Adenine
ABA	Absisic Acid
ANOVA	Analysis of Variance
BA	Benzyl Adenine
bp	Base Pair
cDNA	Complementary Deoxyribonucleic Acid
CIPK23	Cbl-Interacting Protein Kinase 23
CKs	Cytokinins
CTAB	Cetyl Trimethylammonium Bromide
dH_2O	Distilled Water
DNA	Deoxyribonucleic Acid
dNTPs	Dinucleotide Triphosphate
DOG1	Delay of Germination 1

EDTA	Ethylenediaminetetraacetic Acid
EtOH	Ethyl Alcohol
GAs	Giberellic Acids
GGDP	Geranylgeranyldiphosphate
IAA	Indole-3-Acetic Acid
IBA	Indole-3- Butyric Acid
iPBS	Inter Primer Binding Site
K	Potassium
LTR	Long Term Repeat
MEP	2-*C*-Methyl-Derythritol Phosphate
MFT	Mother of Flowering Time
$MgCl_2$	Magnesium Chloride
MS	Murasige-Skoog
$Na_2SO_2O_5$	Sodium Metabisulphite
NaCl	Sodium Chloride
NCED	Nine-cis-epoxycarotenoid Dioxygenases
ng	Nano Gram
Nitrogen	N_2
NOHU	Niğde Ömer Halisdemir University
NTSYS	Numerical Taxonomy and Multivariate Analysis System
P	Phosphorus
PBA	Tetrahydropyranylbenzyl adenine
PBS	Primer Binding Site
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
pH	Potential for Hydrogen
PHYA	Phytochrome A
PIC	Polymorphism Information Content
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SAS	Statistical Analysis Software
TAE	Tris-acetate-EDTA

TE	Tris Ethylene Diamine Tetra Acetic Acid
tRNA	Transfer Ribonucleic Acid
Trp	Amino Acid Tryptophan
U	Units
UPGMA	Unweighted Pair Group Method using Arithmetic Average

CHAPTER I

INTRODUCTION

Ornamental plants are grown all over the world considering their aesthetic and visual characteristics. They offer beauty, attractiveness and aesthetic values to living spaces, moreover they are also economically important. Areas under cultivation of ornamental plants in Turkey is approximately more than 54.128 hectares. In Turkey, ornamental plants are majorly grown in the Aegean, Marmara and Mediterranean regions (TUIK, 2020). Major faction of ornamental plants that are used in landscapes are produced in local nurseries. Furthermore, Turkey also imports a lot of ornamental species from other countries (Haspolat et al., 2016). Turkey is rich in plant diversity as it has its own vast diversity of flora and this potential can be exploited to reduce imports of ornamental plants from other countries (Girmen and Karagüzel, 2005).

Among ornamental plants, the most economically important plant group are the geophytes, they are generally bulbous, rhizomous and tuberous plants (Kesici et al., 2010). The flora of Turkey contains wild forms of many geophyte species that are cultivated and produced for various agricultural purposes (Haspolat et al., 2016). In addition, Turkey's flora is also rich in perennial, biennial, herbaceous and woody plants (Tan, 2010) which can be used as cut flowers, dried flowers, cover plants, grasses, indoors or outdoor plants (Dilbirliği, 2007).

Ornamental plants are indispensable elements of landscape work (Robinson, 1992). Plants which possess aesthetic and functional features are preferred in landscape. Those plants which have attractive and aesthetic features on the basis of their color, texture, form, fruit, flower and seasonal color changes are favored in landscape studies (Özer et al., 2009). Plants can be helpful in preparing habitats for fauna and flora, safety against soil erosion, avalanches, protection from landslides, creating wind curtains, reducing and preventing sound pollution, balancing humidity and temperature, reducing light intensity in cities and creating recreational spaces (Çepel, 1988; Ürgenç, 1998; Walker, 1991; Beckett et al., 1998; Novak and Crane, 2002).

Exploiting natural plant species for landscape use may result in several advantages. Natural species are one of the most important parts of the ecosystem and their study can lead us to ensure healthy continuity of the our ecosystem. Wild plant species highly adapt to biotic and abiotic changes during their life cycle (Morales et al., 2001; Franco et al., 2002; Vignolio et al., 2002; Clary et al., 2004). So, the use of wild species as ornamental plants can also help researchers to fight against changing environmental conditions. Wild plants are also important as they are made up of close relatives in the primary gene pool of many cultivated plant species (Tan, 2009).

Plant gene resources are the most important factors for the continuity of life, but sadly, they are threathened by social, economic and environmental factors in agricultural and biological settings. Plant diversity is being destroyed due to several antagonistic unconscious human decisions, climatic changes, use of agricultural pesticides, increasing population and urbanization, overgrazing and industrialization (Karagöz et al., 2016; Önder, 1997). Thus it is difficult to discover new plant species that have the potential to be used as ornamental plants however, evaluating the available ones for planting studies offer us the most effective way to protect plant gene resources (Helfand et al., 2006). Many human-induced factors have lead to endangerment and extinction of plant species. Many countries are trying to prevent damages to their natural ecology by introducing environmental laws and regulations. However, changes occurring in the climate because of anthropogenic factors are a major problem to tackle since its impacts are difficult to reverse. Drought, one of the consequences of climate change, has now became a considerable problem for Turkey and world. The decrease in the amount of rainfall in Central Anatolia, Southeastern Anatolia and Eastern Anatolia is considered as an indicator that water requirements of living beings cannot be sufficiently supplied or accessed in the near future (Uzunoğlu et al., 2015). Because of the aforementioned reasons, it has become necessary to take measures that require less and efficient use of water for ornamental plants and landscape farming (Çorbacı et al., 2011). Similarly, it is also necessary to apply landscape techniques suitable for sustainable and efficient production (Bayramoğlu, 2016, Güvenç and Demiroğlu, 2016).

It is easy to find plant species that can be considered as ornamental but it is a struggle to identify plants suitable to the changing environmental conditions in Turkey's geography. So, there is a need to carefully choose wild plants that adapt to local

ecological conditions of any region where they are located (Dilaver, 2014). Recently, some studies have been started to identify plant species that have never been used as ornamental plants in Turkey to access their applicability (Gül et al., 2012). Moreover, studies to evaluate those plant species that can be used as ornamental plants, identifying their usability, analysing their functional and aesthetic values and using them for landscaping are increasing rapidly (Arslan and Ekren, 2018; Çimen and Ulus, 2020; Deniz and Yazgan, 2016; Kılıçaslan and Dönmez, 2016; Özdemir and Çiçek, 2017; Erzurumlu and Savran, 2019). In addition to the aesthetic and functional characteristics of the plants that have potential to be used as ornamental plants, their vegetation period should also be taken into consideration (Özdemir and Çiçek, 2017).

Breeding programmes for ornamental plants are being carried out in Turkey and many factors are taken into consideration for this purpose. These factors include resistance to abiotic and biotic stress, fragrance, colour, plant structure, flowering time, longevity and performance after harvest. Even though many experiments have been conducted for development of new cultivars, desired results have still not been achieved (Gülbağ, 2015). Therefore, it is important to include wild plants as breeding materials for breeding of ornamental plants. Cultivation is an important step in the choice of any plant that can be used as an ornamental as it is made up of a long process of seed collection and production of plants. Knowledge about taxonomy, identification, growing conditions, habitat, floristic characters, morphology and post-harvest performance of plants are requirements for the cultivation process (Webber and Johnton, 1998; Grimshaw, 2002). In addition, it is also important to investigate different propagation methods that can be used to develop ornamental plants in nurseries and greenhouses (Karagüzel et al., 2002).

The data obtained on the adaptation capabilities, growing conditions and propagation methods of wild plants from the cultivation process will provide a structure for future works. It will enhance the creation of a more dynamic industry as the demand for wild flowering plants increase. Furthermore, the use of wild plants for landscape designs will raise an awareness and help in the conservation strategies of plant diversity.
The main objective of this study is to ascertain the possibility of using wild flowering plant species for landscape horticulture in semi arid areas like in Niğde.

To achieve this aim, the following were employed;

1. The examination of a variety of wild plant species that have ornamental properties to identify which ones could grow well with little modifications under semi controlled greenhouse conditions.
2. The selection of *Consolida orientalis* (Gay.) Schröd. (Ran) and *Consolida raveyi* (Boiss.) Schrödinger which naturally grow in Niğde, possesses ornamental values and their suitability for landscape arrangement.
3. The propagation of seeds for the investigation of the physiological and morphological characteristics of these species.
4. Seeds were subjected to pre-sowing treatments to observe their effects on germination and seedlings propagation.
5. Molecular studies were carried out using iPBS markers to ascertain the genetic diversity of wild species belonging Ranunculaceae.

CHAPTER II

LITERATURE REVIEW

2.1 Overview of Plant Diversity in Turkey

Turkey is one of the countries which are rich in plant diversity. Turkey's flora is a valuable habitat housing approximately 12.000 plant taxa (Güner et al., 2012; Kaya, 2014). It is known that approximately 4000 species exist in Turkish geography (Erik and Tarıkahya, 2004; Kaya, 2014) and rate of endemism is high (%34.4) (Özhatay et al., 2003). Turkey, has a rich plant diversity as compare to other surrounding countries, especially in terms of endemic species as a result of the floristic richness of the geographical area (Şehirali et al., 2005). Climate varies among small regions, with differences in types of soil, differences in altitute from west to east among other factors (Demir, 2015). These factors have led to the diversification of plant species that make up plant populations and also influence the location of endemic plant species (Avcı, 2005; Demir, 2015). Besides the geographical factors, two of the Vavilov's Center of Origins which are Near Eastern and Mediterranean Centers cover parts of Turkey (Tan, 2009). This makes Turkey one of the important Centers of Origin and/or Center of Diversity for many wild and cultivated crops in the region. The exposure of Turkey to different civilizations and its central position in the migration routes during history contributed to the diversity in Anatolia. These factors have contributed to diversification of plant and gene pool (Demir, 2015). The existence of rich plant populations in Turkish flora is closely connected with the phytogeographical regions that overlaped in the Anatolian region (Figure 2.1). The phytogeographical regions include Euro-Siberian, the Mediterranean and Irano-Turanian which show variations in terms of ecological factors of Anatolia. Flora of Irano-Turanian and Mediterranean region show high endemism rate.

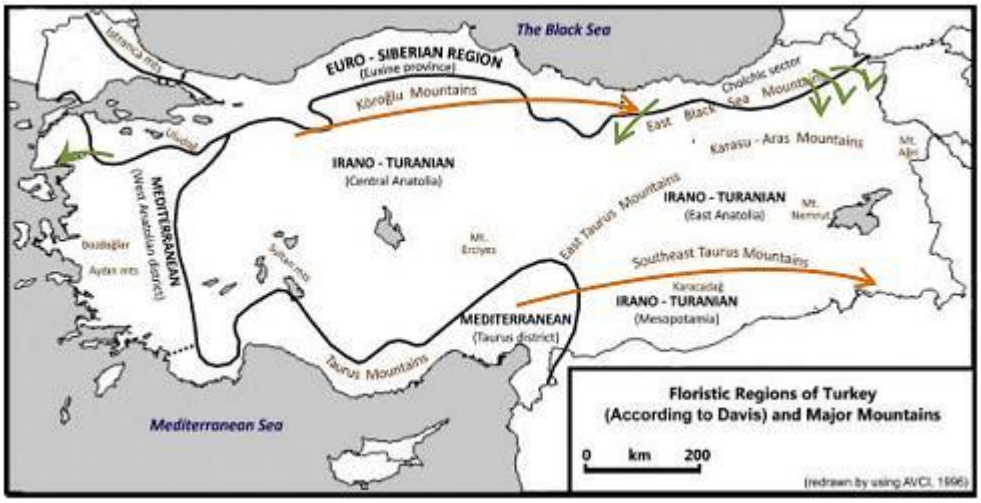

Figure 2.1. Phytogeographic regions of Turkey (Köse et al., 2012)

In addition, five micro centers of diversity (Trakta-Ege, Güney-Doğu Anadolu, Samsun-Tokat-Amasya, Kayseri-area and Ağrı-area) are also in Turkey. These micro centers consist of wild relatives of many vegetables, fruits and cereal species (Açıkgöz et al., 1998; Demir, 1990; Demir 2015; Harlan, 1995; Gönülşen, 1986; Tan and Taşkın 2009, Tan, 2009). Turkey's plant diversity is not only limited to these centers, it also includes the plant populations that are grouped in plant genetic resources including medicinal and aromatic plants (Karık et al., 2016) as well as ornamental plants (Haspolat et al., 2016).

Turkey has a rich biodiversity of ornamental plants due to its topography and climate. Many wild plants can be utilized as ornamental plants such as geophytes, woody and herbaceous perennials, biennials and annuals (Tan, 2009). Geophytes are the most attractive plants in this group. Therefore, many geophytes were collected from Turkey (habitat) and used by foreign researchers as breeding material (Kaya, 2014). Wild flower bulbs such as *Cyclamen*, *Galanthus*, *Leucojum*, *Anemone*, *Eranthis*, *Fritillaria* and *Lilium* have been exported from Turkey to other countries for ornamental purposes. Several herbaceous plants with ornamental features are found in the Turkish flora examples of which include; *Campanula, Dianthus, Geranium, Iris, Narcissus, Salvia, Tulipa, Verbena*, *Veronica* and *Viola* genus (Akan and Tay, 2006).

The Turkish vegetation has suffered some damages in time past (Tan, 2010). After this experience, survey and collection of ornamental plants for conservation as breeding

materials started in Turkey in 1964. The first step of conservation strategies consisted of determining distribution areas of plant species and collecting them. There are two kinds of conservation startegies for plant species that have potential to be used as ornamental plants. These are the *ex situ* and *in situ* conservation methods used in managing plant species. Annual or perennial herbaceous plants propagated by seed are conserved in seed genebank (*ex situ*), on the other hand geophytes and perennial woody plants are conserved in the field or in their natural habitat (*in situ*). Vegetative and generative materials of many plant species can be found under conservation in the National Gene bank at Aegean Agriculture research Institute (AARI) in Turkey (Haspolat et al., 2016; Tan, 2009). Plant diversity is protected by following conservative rules and regulation of Turkey regarding species (Tan, 2009). Improvement in agricultural production and the introduction/conservation of new plant resources is essential for national and international trade (Tan, 2009).

2.2 Assesment of Wild Plant Species as Ornamental Plants

The description of "wild" can be made as the capacity of emerge (semi) naturally in a location or independently through human enforced external factors. (Heywood, 1990). Wild plants are sources that can be utilized for ornamental purposes. Even though they are not produced for commercial purposes, they can play important roles in the flower industry (Pascale and Romano, 2017). Nature presents many wild flowers that possess aesthetics features. Futhermore, maintenance of wild flowers does not require labor as compared to cultivated ornamental plants (Sulistyantara and Mentari, 2017). Wild plants have mostly been prefered as ornamentals. This situation results in uniformity in terms of landscaping (Younis et al., 2009). Therefore, effect of uniformity due to maintenance problems in landscaping can be overcomed by using wild plants as new ornamentals (Pascale and Romano, 2017).

Wild plant species are adapted to environments by changing color, texture, shape and other characteristics depending on seasonal changes and their development stage (Özdemir and Çiçek, 2017). Moreover, wild plants are also important mediators in conserving the ecological stability in nature like their less demand for water, pesticides. Preference of wild plants as ornamental plants provide chances of conservation for plant genetic diversity (Pascale and Romano, 2017). Wild plants that can be used as

ornamental plants need to be determined first in a group. The real potential of wild plants could not be well exploited due to lack of planning and information about their adaptation to new enviroments. The cultivation of a wildflower requires proper understanding of all the conditions that will ensure the healthy growth of that flower in a particular region. Many works are still on-going to learn more about these wildflowers for their various implementations particularly for landscape usage (Younis et al., 2009).

Many studies have been conducted to determine wild plant species having possibilities to be used for landscaping design as new ornamental plants. Özdemir and Çiçek (2017) reported that 12 wild plant species have been determined to be utilize as ornamental plants by measuring their morphologic characteristics, flowering time, life cycle and ecological properties in a research area. Kılıçarslan and Sönmez (2016) conducted a research on bulbous plants in Lakes region and suggested that many bulbous plants naturally growing in this region. The areas where plants can be used in landscaping have been exhibited and a flowering calendar has been generated for bulbous plants. Gül et al. (2012) reported that ground cover plants have been grown naturally in Isparta region. Adaptation studies have also been conducted on ground cover plants revealing the identity of some species that can be used as ground cover. Erzurumlu and Savran (2019) indicated that wild plant species have been investigated in the campus area of Nigde Ömer Halisdemir University (NOHU), ecological and morphological properties of wild species have been considered for study and some favorable wild plants have been found as a valuable source that can be used for landscaping designs. Haspolat et al. (2016) found out 66 families that can be used as ornamental plants in different provinces of Turkey. Some of the plant families include Fabaceae, Asteraceae, Lamiaceae, Rosaceae, Ranunculaceae, Liliaceae, Violaceae, Campanulaceae, Solanaceae, Iridaceae, Caryophyllaceae and Malvaceae having ornamental value.

2.2.1 Ranunculaceae family

Ranunculaceae family is represented as a large diversity due to their cosmopolitian distribution (Tamura, 1993). Although they can be found in various habitats (Tamura, 1993), their center of origin is the cold and temperate zone of the Northern and Southern Hemisphere (Heywood et al., 2007). The family has about 43 genera and 2346 species. 204 species belonging to 20 genera of Ranunculaceae exist in Turkey. The

number of endemic plants in Turkey are 51 (Güner et al., 2012; Christenhusz and Byng, 2016). The members of Ranunculaceae consist of terrestrial or aquatic, annual or perennial shrubs, herbaceous and climber plants (Ertuğrul et al., 2012). The morphological characters (leaf, flower and fruit) of Ranunculaceae are wide in variation (Emadzade et al., 2010). The members of Ranunculaceae generally have protandrous flowering but rarely protogynous. For this reason, fertilization occurs by cross pollination and it helps to increase genetic diversity (Heywood et al., 2007). Many genera of Ranunculaceae family are used as ornamental plants for landscaping due to their colourful and beautiful flowers. Examples of which are, *Aconitum, Adonis, Anemone, Aquilegia, Caltha, Cimicifuga, Clematis, Consolida, Delphinium, Eranthis, Helleborus, Hepatica, Nigella, Pulsatilla, Ranunculus, Thalictrum* and *Trollius* (Tamura, 1993).

2.2.1.1 *Consolida orientalis* (Gay) schröd. (Ran)

Consolida orientalis is a specie which belongs to Ranunculaceae family in taxonomic classification. It is a herbaceous annual plant specie with a stem reaching a height of 20-74 cm and displays simple and branched structure. The infloresence consists of dense raceme and intense violet-coloured flowers. Flowers contain spur which is shorter than pedicel. Bracteoles reach the base of flowers and are attached to the upper part of pedicels. Leaf arrangements on the stem is alternate and leaves emerge from each node. Leaves are laciniae numerous and linear-setaceous. Flowering periods are during May through to August. Habitat is grain and fallow fields while altitude of species is between 0-1900 m (Davis, 1965; Güner et al., 2012; Tekin, 2007). The taxonomic classification of specie *C. orientalis* is outlined below (Anonymous, 2021a).

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Magnoliidae
Order	:	Ranunculales
Family	:	Ranunculaceae
Genus	:	*Consolida*
Species	:	*Consolida orientalis* (Gay) Schröd. (Ran)

2.2.1.2 *Consolida raveyi* (Boiss.) schrödinger

Consolida raveyi is a species that belongs to Ranunculaceae family in taxonomic classification. *C. raveyi* is endemic and a herbaceous annual plant species with simple stems and sparingly branched structure. Plant height reaches 10-40 cm, infloresence is raceme which is spike-like. Their flowers are violet in color and do not have pedicel, moreover leaves are laciniae linear or linear-oblanceolate adpressed-pubescerit. Flowering occurs during the months of June and July. Their habitat is steppe, stony places, fields and altitude is between 900-1600 m (Davis, 1965). The taxonomic classification of specie *C. raveyi* is outlined below (Anonymous, 2021b).

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Magnoliidae
Order	:	Ranunculales
Family	:	Ranunculaceae
Genus	:	*Consolida*
Species	:	*Consolida raveyi* (Boiss.) Schrödinger

2.3 Propagation of Wild Ornamental Potential Plants

Ornamental horticulture can help in raising awareness for wild plants as potential candidates to be used in landscape design and as alternative sources for floriculture (Mayer et al., 2017; Pascale and Romano 2017). It is a primary requirement to carry out studies on wild plant production (Dilaver, 2013). A major feature of the ornamental plant industry is its diverse nature. Presently, the cultivation of ornamental plant species is larger than a combination of both agricultural and horticultural crops (Weiss, 2002). Plant production mechanisms for cultivation in different other regions in addition to their natural habitats are required not only for the purposes of conservation and multiplication but also to enhance the large scale propagation of the plants (Gurbuz et al., 2009). Plant propagation is very important because of the way it enhances fast multiplication of plants without any effect on the desirable traits found in the mother plants. In attending to propagation difficulties or optimizing nursery production of species, various techniques apply to different species. Plant propagation is basically carried out either sexually (by seed) or asexually (by vegetative parts). In nature, however, plant propagation is mostly by sexual reproduction hence several species are sexually reproduced. Horticulturists over the years have explored and developed asexual reproduction methods that support vegetative plant parts for propagation purposes (Roberto and Colombo, 2020). Several studies regarding propagation of wild plant have been conducted in this area in recent years (Ari et al., 2014; Dilaver, 2013; Gurbuz et al., 2009).

2.4 Importance of Seed

Biologically, the seed is one of the main products of a plant. Seed is a propagation material which enables the continuity of plants (Sharififar et al., 2015). They contain the genetic make-up of their parent plants with the potential of adaptation to environmental changes. Seed is the main tool that carry genetic variation from present to future generations (Boswell, 1961). History of seeds dates back from ancient times for agricultural production (Raikwar, 1975). Farmers started to select seeds of desirable wild crops for their food and those species started to be domesticated. So, the demand for quality seeds by farmers increased and it became an essential component for researchers over time (Bareke, 2018).

2.5 Seed and Seed Development

The seed is the primary material for propagation of vascular plants. Seed is produced by plants both sexually and asexually. Sexually, seed production occurs through fertilization (Sliwinska et al., 2014). During seed development, numerous crucial events take place from fertilization to seed maturation. The pollen which is the male part of a flower and the embryo sac the female part play a major role in fertilization. Flowering plants produce healthy pollen grains, these pollen grains on reaching the surface of the stigma start to germinate resulting in the formation of pollen tubes. The pollen tube has two sperm nuclei (n), the generative and tube nucleus, which elongates into the style until it reaches the ovary. The ovary has the embryo sac that contains three antipodal cells (n), two synergids (n), one polar nuclei (n) and an egg (2n). Fertilization takes place in the embryo sac where fusion of two sperms occur with the egg and polar nuclei. Fusion of one sperm and egg (2n) forms embryo (2n),whereas the other sperm and polar nuclei constitutes the endosperm (3n). This whole process is called embryogenesis (Bareke, 2018). After embryogenesis, ovules form mature seeds (Haughna and Chaudhuryb, 2005).

Seeds are made up of three parts which are, the seed coat, endosperm and embryo. The seed coat develops from the integuments which is a layer that surrounds the nucleus present in the ovule. The integument can be either one or two in the Gymnosperms and Angiosperms, respectively (Bareke, 2018). The seed coat is a barrier which protects the inner content of the seed which are the endosperm and the embryo (Finch-Savage and Leubner-Metzger, 2006) from the effects of biotic stress, mechanical injury and against dehydration. It also helps in gas exchange and water uptake for germination. Dispersal of seeds can be done by animals, mainly birds due to the attractive color of seed coats in some plants (Sliwinska et al, 2014). The other part of the seed which is the endosperm, is formed as a result of fertilization, it is the main source of food i.e. fats, carbohydrates or proteins (Yamada et al, 2001). It also provides energy to the embryo during germination to produce healthy plantlets. Besides, the size of the endosperm can vary from specie to specie during the seed maturation stage. The embryo is the primary structure that allows a new plant to geminate. A mature embryo is made up of an embryonic axis in angiosperms having one cotyledon (monocot) or two cotyledons

(dicot). Radicle, hypocotyl, epicotyl and plumule are parts of embryonic axis. After germination, an embryonic root develops from the radicle that contains root meristem. The hypocotyl is the part between radicle and cotyledon of seedling. The epicotyl is the first shoot segment above the cotyledon (Sliwinska et al., 2014).

Hormonal regulation in plants control seed development and maturation (Black 1991; Khan 1982). Hormones such as cytokinins, auxins, gibberellins and abscisic acid have the potential to alter the expression and activitiy of seed development and maturation (Rock and Quatrano, 1995). In addition, some environmental components affect seed development and maturation after fertilization, these environmental factors include soil fertility, water, temperature and light. The amount of chemicals such as nitrogen (N), potassium (K) and phosphorus (P) has associated impacts on seed size during seed filling and promotes seed development. Among these elements, N is the most responsible chemical for enhancing seed size, seed germination and vigor (Bareke, 2018). Seed development is hindered under water deficiency as it negatively affects seed size. Number of seeds in a plant are also affected by lack of water during the flowering stage. Small seeds develop under high temperature while seed growth seizes under low temperature. Light is a major source in plant development, if plant is exposed to lower solar radiation, plant growth is negatively affected and this leads to small seed size and limited number of seeds produced (Copeland and McDonald, 2001).

2.6 Seed Germination

Seed germination is the most crucial stage in the establishment of healthy seedlings. Abiotic and biotic stresses are the most significant factors in plant life which causes economic and ecological problems in agriculture (Nonogaki et al., 2010; Tuan et al, 2019). In short, germination is a critical physiological phase that enables reproduction in plants.

Dry seed is seed in an inactive form before the germination process, some metabolic, molecular and chemical changes take place during germination which is presented in Figure 2.2 (Nonogaki et al., 2010; Tuan et al. 2019). First stage of germination is imbibition, where dry seeds start absorbing water through the seed coats. Radicle

penetration starts with the onset of metabolic activities in seed (Bewley, 1997; Tuan et al., 2019; Weitbrecht et al., 2011).

The water uptake in seed takes place in three phases (Bewley, 1997; Tuan et al., 2019), during Phase I, water is taken from the seed coat and the seed begins to swell. The membrane structure is destabilized by hydration and leads to ion and solutes leakege from the seed and the membrane recovers shortly after the hydration process (Bewley, 1997; Tuan et al., 2019). During the first phase, many physiological processes occur including protein synthesis and increase in respiration metabolism. Damaged DNA which is caused by loss of water during seed maturation is reformed by the uptake of water (Bewley, 1997; Tuan et al., 2019; Weitbrecht et al., 2011). Imbibition also affects the construction of mitochondria which was in less amount in dry seeds. Increase in the amount of mitochondria via seed imbibition helps synthesis of ATP needed for germination (Rajjou et al., 2012). Later, Phase II starts with the absorption of water wind down and stabilized. A set of biological events that occur in the first phase continue to renew DNA and mitochondria during the second phase. Moreover, new gene transcripts start to develop and they contribute to protein synthesis (Bewley, 1997; Nonogaki et al., 2010; Tuan 2019). Phase III is called the post germination stage which sees the emergence of radicula from the weakened seed coat from the second phase. This is the complete germination process in those seeds which do not require dormancy (Tuan et al., 2019). Figure 2.2 describes all three phases during germination.

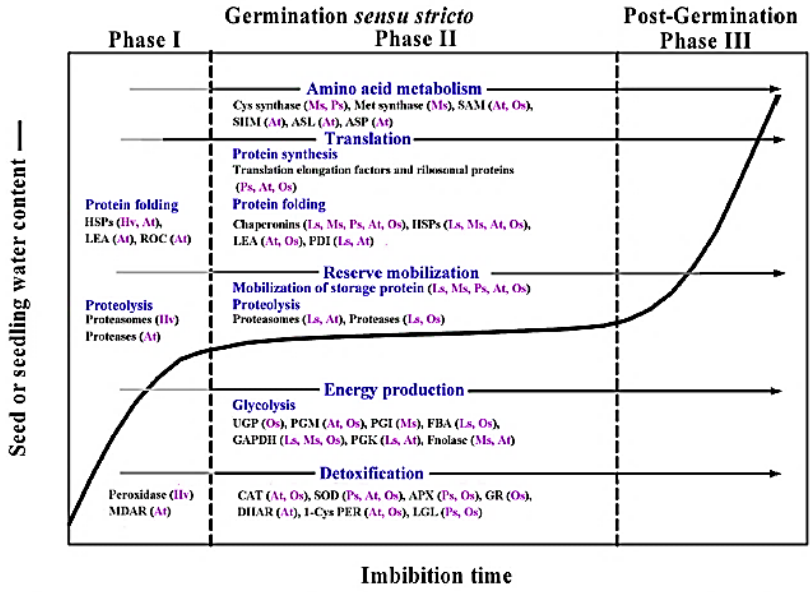

Figure 2.2. Some biological changes during seed germination (Wang et al., 2015)

2.7 Seed Dormancy

Non-dormant seeds germinate quickly under favorable conditions of adequate water, appropriate temperature, oxygen and light (Rifna et al., 2019). However, if germination is prevented by unfavorable circumstances, and under optimal environmental conditions we have a situation known as dormancy (Tuan et al., 2019). It is also called resting stage of seed (Figure 2.3) (Bareke, 2018).

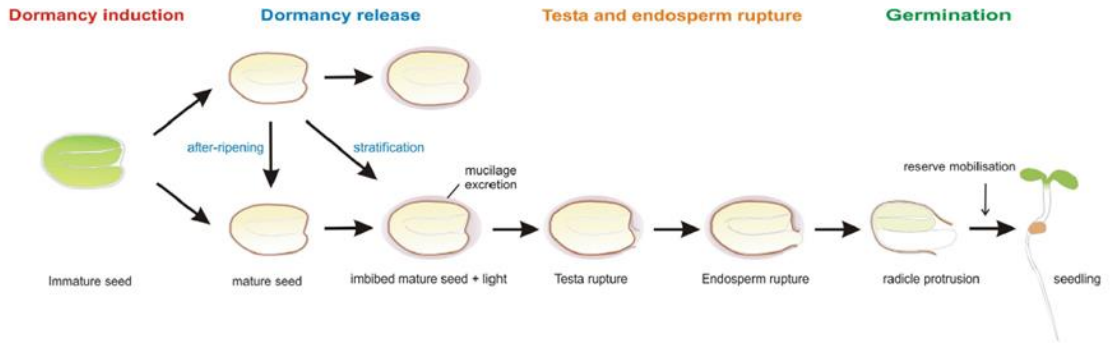

Figure 2.3. Germination process of dormant and non-dormant seeds (Bentsink and Koornneef, 2008)

There are two types of dormancy; primary and secondary (Figure 2.4). In non-dry seeds, (newly collected seeds) there is high content of ABA which causes primary dormancy (Finch-Savage and Leubner-Metzger, 2006). Primary dormancy was categorized

physiologically and morphologically by Marianna G. Nikolaeva (Nikolaeva, 1967, 2004). Further categorization was proposed by Baskin and Baskin (2004). Depending on the classification, primary dormancy splits up into five groups which are physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dormancy, and combinational dormancy (physiological and physical dormancy) (Tuan et al, 2019). Physiological dormancy is the most common dormancy in many plants, particularly including weeds. In case of the dormancy level, the physiological dormancy is divided further into three groups; nondeep, intermediate and deep dormancy. Healthy seedlings are developed from seeds that exhibit nondeep dormancy, on the other hand abnormal seedling production or growth problems occur in deep dormancy plants. Underdeveloped embryos induce morphological dormancy (Heather et al., 2010; Tuan et al., 2019). Seed coat inhbits water uptake and water exchange in physical dormancy during germination. Physical and physiological compounds of the seed causes combinational dormancy (Tuan et al., 2019).

Non-dormant seeds can suffer from dormancy due to unfavorable conditions. Therefore, germination of imbibed non-dormant seeds can be delayed and extended for a while even under favorable conditions. This condition is called secondary dormancy. Seasonal factors are the determinants of secondary dormancy. Maintenance of secondary dormancy is to responsibly manage temperature under the field conditions (Buijs, 2020). This phenomenon has positive correlation with soil seed bank and it also conserves the welfare of plant population (Ayuso et al., 2019).

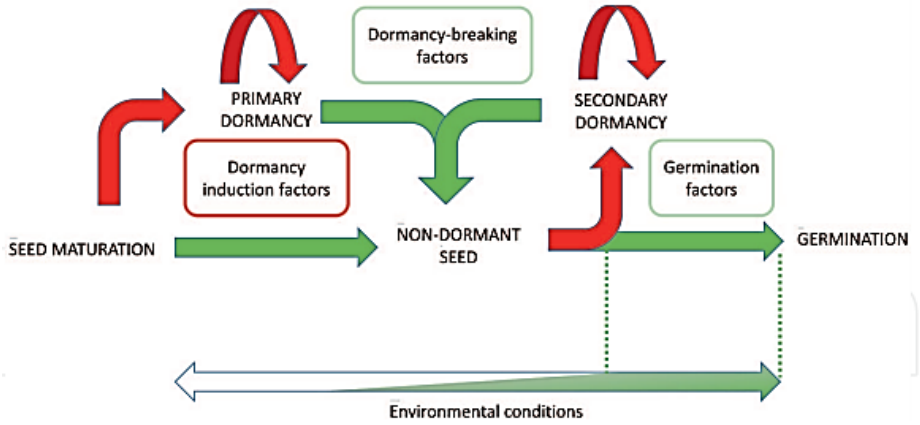

Figure 2.4. Environmental factors influencing the seed germination and dormancy, Red arrows shows the factors involved for inducing dormancy of seed, whereas green arrows depicts the normal process of germination. Normal germination process continues when seed fulfil s the desired conditions by overcoming the dormancy factors as shown with green arrows (Ayuso et al., 2019)

Hormones which are synthesized during seed development, are responsible for seed dormancy and germination (Bentsink and Koornneef, 2008; Gao and Ayele, 2014). Most important hormones are Absisic acid (ABA) which promote dormancy whereas Giberellic acids (GAs) initiates seed germination. The other hormones that also impact on dormancy and germination include ethylene, cytokinins, brassinosteroid, auxin, and jasmonates (Arc et al., 2013; Beaudoin et al., 2000; Corbineau et al., 2014).

Dormancy is controlled by hormonal regulation of ABA which is a member of isoprenoids (Figure 2.5) (Nambara, 2010; Tuan 2019). The rate of ABA increases during two stages, first during embryo development and secondly during formation of zygotic tissue (Karssen et al., 1983; Koornnef et al., 1989). Maternal tissues play a role in the production of ABA (Karssen et al., 1983; Kanno et al., 2010) which also contributes to the induction of embryo development (Frey et al., 2012; Raz et al., 2001). On the other hand, ABA derived from zygotic tissues can cause seed dormancy (Karssen et al., 1983; Koornneef et al., 1989). Some metabolic genes impact on biosynthesis and catabolism of ABA. Some members of *NCED* (Nine-cis-epoxycarotenoid Dioxygenases) and *CYP707A* gene families (Lefebvre et al., 2006; Frey et al., 2012) are also associated with the ABA biosynthesis and catabolism in plants (Nambara, 2010). *NCED5*, *NCED6* and *NCED9* genes are responsible for the regulation of ABA and trigger dormancy (Cadman2006; Lefebvre et al., 2006; Frey et al., 2012). *CYP7072* is member of *CYP707A* gene families which are responsible for the

catalytic activity of ABA during seed germination (Figure 2.5) (Footitt et al., 2011, Millar et al., 2006).

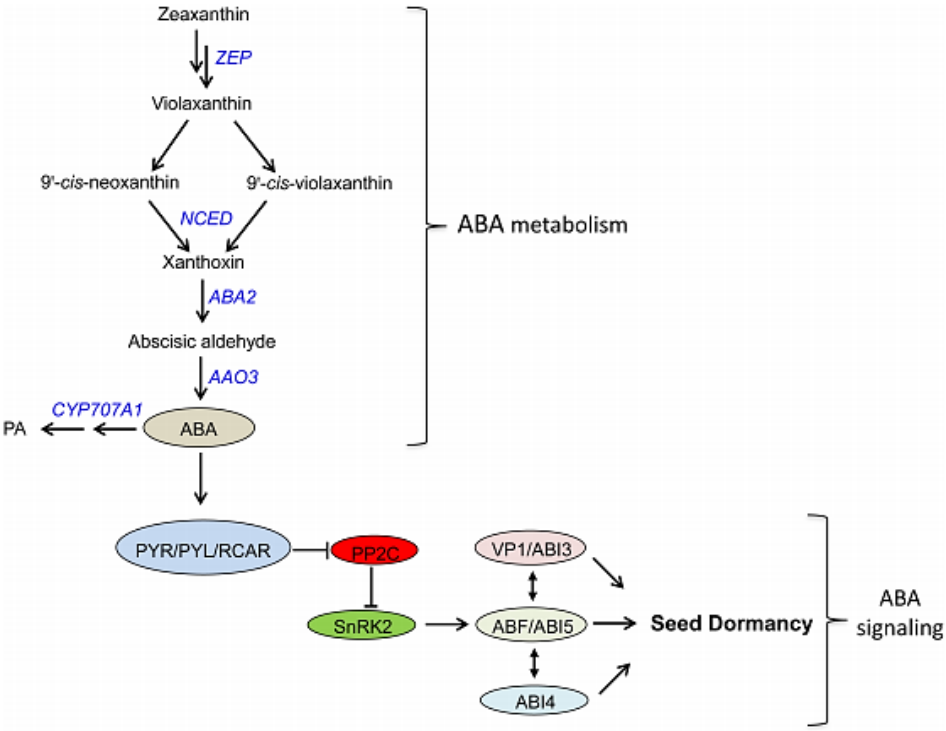

Figure 2.5. Flow chart diagram showing the metabolism of abscisic acid along its signaling pathway for the intiation and maintenance of seed dormancy (Tuan et al., 2018)

Apart from ABA, another hormone that control seed germination is GAs (Figure 2.6). (Yamaguchi et al., 2018). GAs are a member of diterpenoids that enhance germination of seed and supports plant growth. It also helps to break dormancy of seeds (Tuan et al., 2019). Numerous GAs have been discovered in plant fungi and bacteria (Hedden and Sponsel, 2015). Bioactive form of GAs are GA_1, GA_3, GA_4 and GA_7 (Tuan et al., 2019). The 2-*C*-methyl-derythritol phosphate (MEP) pathway synthesizes geranylgeranyldiphosphate (GGDP) which is a precusor compound for biosynthesis of GAs (Milborrow and Lee, 1998; Hirai et al., 2000; Kasahara et al., 2004). *GA20ox*, *GA3ox*, and *GA2ox*, are members of multigenes family, associated with GA metabolic patways (Tuan et al., 2019). *GA3ox1* is the responsible gene for biyosynthesis of GA and helps in seed germination, however *GA2ox2* gene induces catabolism of GA and causes seed dormancy (Figure 2.6) (Footitt et al., 2011; Graeber et al., 2012).

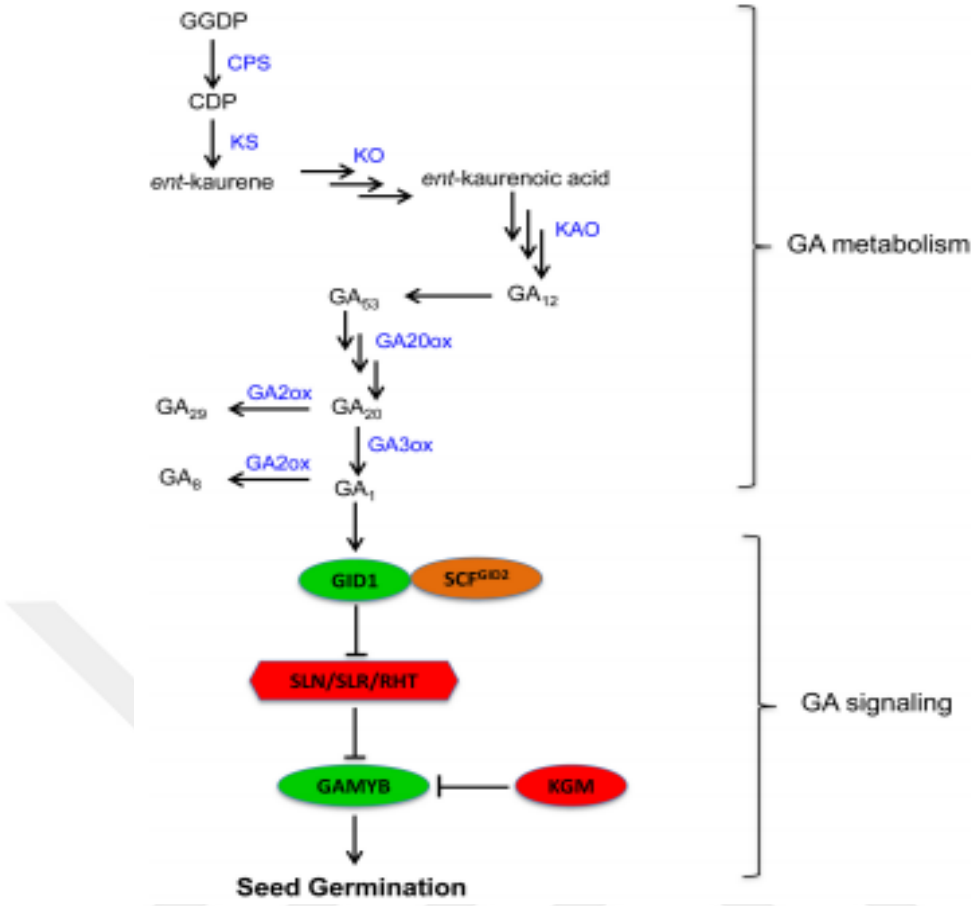

Figure 2.6. Flow chart diagram showing the metabolism of gibberellin along its signaling pathway for the intiation of seed germination (Tuan et al., 2018)

Regulation of seed dormancy and germination is mainly under the control of GA/ABA balance in monocots and dicots (Tuan et al., 2019). On the other hand, other types of phytohormones also participate in the control of seed dormancy and help in germination (Shu et al., 2016; Tuan et al., 2019). Cytokinins (CKs) are phytohormones and Isopentenyl Pyrophosphate Transferase (IPT) enzyme that is responsible for biosynthesis from adenine molecules (Kakimoto 2001; Murai 2014; Takei et al., 2001). Several CKs are known to be isolated until today (Algül et al., 2016). First isolated CK is zeatin from immature maize kernels (Lennarz and Lane, 2013). Zeatin and 2IP (isopentenyl adenine) are natural forms of CKs wheras, kinetin (N6 furfurilamino purine), BA (benzyladenine) and PBA (tetrahydropyranylbenzyl adenine) are the synethetic types of CKs (Algül et al., 2016). CKs are functional during seed germination processes (Miransari and Smith, 2014). CKs shows antogonistic effects on ABA through down-regulating *ABI5* transcription factor to stimulate seed germination (Wang et al., 2011b). Accumulation of CKs mainly occurs in the liquid endosperm

during seed development stage (Emery et al., 2000; Kucera et al., 2005). Besides, CKs also impact on seed size, embryo, endosperm and testa growth (Mansfield and Bowman, 1993; Miransari and Smith, 2014). CKs are also synthesized in roots (Kumlay and Eryigit, 2011) and play a role on the meristematic cell differentation of this tissue (Murai, 2014). Transportation of CKs occur through xylem and phloem vessels after synthesis in the root to other parts of the plants (Murai, 2014). CKs play a role in many developmental processes particularly in the growth, development and senescence of leaf and host-microbe interactions (Akhtar et al., 2020). CKs have the ability to moderate effects of abiotic stress factors on seed germination (Atici et al., 2005; Mirensari and Smith, 2014; Peleg and Blumwald, 2011).

Auxin is another type of phytohormone that is necessary for setting some cellular processes like seed development in place (Cao et al., 2020). Although the presence of auxin is not required for germination, auxin is majorly located in radicle tip during and after seed germination as a result of investigation of genes which are responsible for expression of this hormone (Liu et al., 2007b). Cellular machinery plays a role in auxin synthesis and transfer and this process has contributed to the development of the endosperm and testa. Therefore, biosynthesis of auxin is important for formation of integuments (Matilla, 2020). The high abundance of free-auxin and metabolites during various developmental stages, both early and late phases, claim that auxin has prominent roles in signaling cascade (Bernardi et al., 2012; Chen et al., 2014; Matilla 2020). In addition, there is a positive correlation between auxin and ABA on seed dormancy (Liu et al., 2013). Besides the effect on the reproductive part of plant, auxin drives many metabolic processes affecting plant growth and development (Bernardi et al., 2012). An active and natural form of auxin is IAA (indole-3-acetic acid). IAA is synthesized from Trp (amino acid tryptophan) through a complex metabolic process in higher plants (Mano and Nemoto, 2012; Zhao 2010). In addition, IBA (indole-3- butyric acid) is a type of auxin. It is known that IBA is a synthetic form of auxin (Zimmerman and Wilcoxon, 1935) but IBA is considered as an auxin precursor and naturally found in many plants (Korasick et al., 2013). IBA has diverse effects on plants during the development of roots (Frick and Strader, 2018).

Besides, dormancy cycling is regulated via some genes that are *DOG1* (Delay of germination 1), *MFT* (Mother of flowering time), *PHYA* (Phytochrome A) and *CIPK23*

(Cbl-Interacting Protein Kinase 23) (Finch-Savage and Footitt 2017). *DOG1* is responsible for initiation of dormancy during seed development period (Dekkers at al., 2016).

Soil temperature and moisture content also have impact on seed dormancy after sowing seeds under field conditions. Therefore, favorable soil conditions are essential for germination of seeds, otherwise seeds remain in dormant phase due to unfavorable conditions (Footitt and Finch-Savage, 2011). ABA level increases with the decrease in soil temperature during winter seasons. Expression of *DOG1* gene increases at the same time and dormancy remains constant in seeds. With the increase in soil temperature, seed dormancy can be decreased during fall season with a higher ABA level and suppressed expression of *DOG1* (Figure 2.7). *MFT* also shows similar functioning like *DOG1* by sensing seasonal changes and increases the expression level and regulate seed dormancy (Finch-Savage and Footitt, 2017).

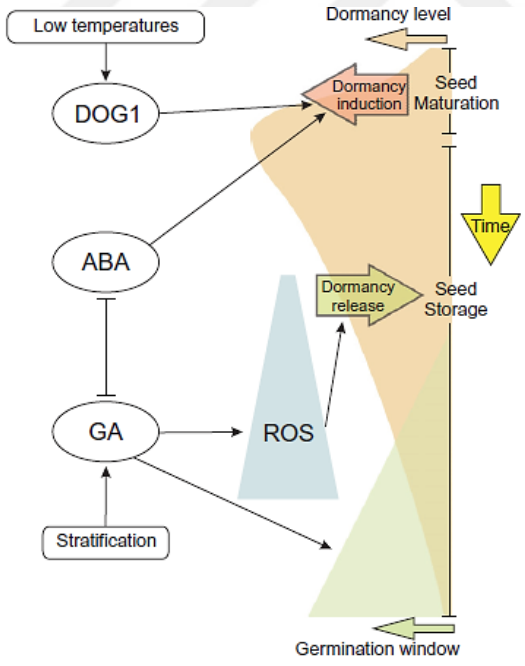

Figure 2.7. Role of *DOG1* gene fo the intiation of seed dormancy, lower temperature favors the transcription of *DOG1* gene (Graeber et al, 2012)

Seed germination and dormancy are the main factors for seedling emergence and robust growth of plant throughout its life cycle. ABA and GA has antagonistic effect on seeds and controls seed germination. Uniform germination and healthy seedling emergence is

important for maintenance of plant productivity and sustainability of plant diversity. Therefore, it is necessary to investigate the pre-sowing treatment before sowing.

2.8 Pre-sowing Seed Treatments

Pre-sowing seed treatments are beneficial to improving seed germination in plants. It helps to break dormancy of seeds and trigger sprouting. These treatments are conducted under control conditions before onset of physical and physiological processing of seeds (Coolbear, 1991). The environmental requirements change based on plant species needed for seed germination. Germination of seed varies from species to species as wet and cold conditions are necessary for seeds of certain plant species while high temperature and dry conditions are important for the others. Therefore different methods are used for achieving higher germination. Seed priming and cold stratification is consider as most common pre-sowing treatments (Coolbear, 1991; Karlsson and Pahlevanyan, 2014).

2.8.1 Seed priming

Sowing time and germination of seeds constitute the most important stage of cultivation which is crucial for plants propagated by seed (Karakurt, 2010; Yıldız, 2007). Although optimum levels of the internal and external factors available for germination and seed germination decreases by various other reasons (Karakurt, 2010). Therefore, to overcome this problem, seed priming a suitably cheap and easy to achieve technique has been investigated to minimize the impact of those factors on seed germination.

Seed priming is a process involving the use of hydration techniques in the treatment of seeds before sowing them in order to enhance their germination (Pill, 1995). The seeds are initially pre-treated in water or in an osmotic solution. Root formation is prevented from the seed coat with seed priming techniques. The treated seeds are then rewashed and left to dry before planting.

Seed germination is classified into three phases, phase I, II and III under most favourable conditions. In the Phase I (Imbibition) is where seeds start uptaking water from the cell membrane. Phase II (Activation) is the reactivation process which is

associated with metabolic and physiological changes. Cell growth starts and finally ends in the protrusion of root in Phase III. Phase I and II can be improved by seed priming (Figure 2.8). Phase III is not passed in order to handle root protrusion from seed layers (Dalil, 2014; Lutts, 2016). Seeds are kept in phase I and phase II before sowing to enhance the effect of these phases on germination (Lutts, 2016).

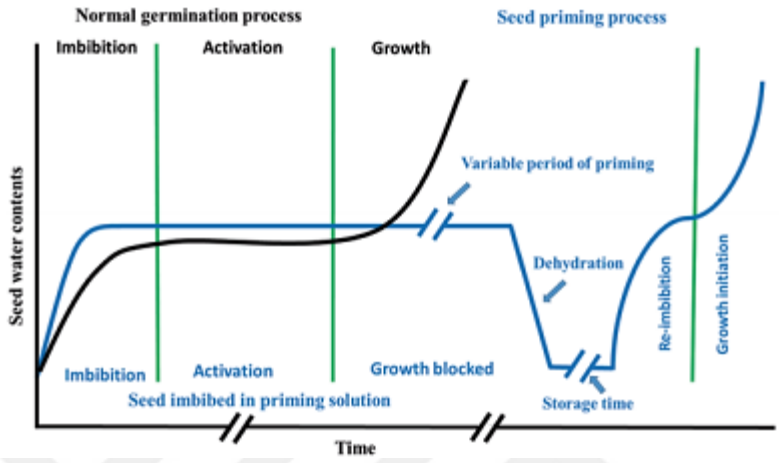

Figure 2.8. Seed priming process (black line represents normal germination process whereas blue line represents seed priming process) (Ibrahim, 2019)

Many studies have been reported in relation to the advantages of seed priming in vegetables, floriculture and some field crops (Toklu et al., 2015). It helps to break dormany for some plant species (Tahaei et al., 2016) and to increase seed vigor (Varier et al., 2010) and to enhance rapid and homogenous germination (Afzal, 2016). It also increases tolerance to abiotic and biotic stress conditions during plant growth (Jisha,2013; Pirasteh-Anoshe and Hashemi, 2020). Primed seeds, enhance the seed performance against stress conditions during germination and at other plant growth stages. Primed seeds germinate faster and have their stress-response systems promptly activated. Priming memory or stress imprinting mechanisms are processes used to register the genetic or biochemical changes induced as a result of the seed priming. These processes enhance a more tolerant response of plants to stress conditions and further helps in raising plants tolerance to subsequent stress exposures (Chen and and Arora, 2013). In a research conducted by Kubala et al. (2015b) primed rape (Brassicanapus) seeds showed tolerance to salinity stress in the germination phase after being exposed to the stress condition. This ability can be attributable to the "priming memory" process.

Different factors influence the efficiency of seed priming such as aeration, light, temperature, treatment duration, seed quality (Dawood, 2018), plant species/genotype and type of priming techniques (Paparella et al., 2015). Several types of priming techniques have been reported using natural and synthetic compounds as agents (Masondo et al., 2018). Water is used as an agent for the seed soaking process known as hydropriming (Kaya et al., 2006). Osmopriming prevents excessive uptake of water to avoid oxidative effect of reactive oxygen species (ROS) on cells by using PEG, sugar, mannitol, sorbitol and inorganic salts as priming agents (Agas, 2019). Seeds are treated in solutions prepared by using inorganic salts for halopriming (Karim et al., 2020). Vermiculite, sand and clay (Paparella et al., 2015; Di Girolamo and Barbanti, 2012) are preferred as priming agents for solid matrix priming to soak seeds. Nutripriming is the soaking of seeds in macro and micro nitrutients (Farooq et al., 2012; Imran et al. 2013). Seeds are innoculated with beneficial microrganisms known as bio-priming (Waqas et al., 2019). Nanoparticles are used as priming agent to soak seeds for nanopriming (Khalaki et al., 2020). Seeds are submerged in solutions prepared by using plant growth regulators for priming (Waqas et al., 2019).

2.8.1.1 Priming with plant growth regulators

Plant growth regulators (PGR) are used in priming techniques. PGRs directly affect metabolic activities during seed germination. A group of PGR that are abscisic acid, gibberellins, auxins, kinetin, salicylic acid, ethylene and polyamines which are mostly preferred as priming agent (Luts, 2016, Waqas et al., 2019). Effects of PGRs in priming has been observed to increase seed germination and vigor under unfavorable environmental conditions such as salinity (Sedghi et al., 2010), drought (Ansari et al., 2013), heavy metals (Espanany et al., 2016), low (Tiryaki and Büyükcingil, 2009) and high tempetature (Akman, 2009).

Effects of Giberellic acid (GA_3) has also been reported for improving seed germination and promotion of plant growth (Dawood, 2018). GA_3 has important roles in the synthesis of hydrolase enzymes. It helps to take some nutritions from the endosperm to the embryos by activating the hydrolase enzymes during the germination process (Abu-Muriefah, 2017). Additionally, it enhances the production of amylase, protease, and glucanase enzymes that are necessary for germination (Yamaguchi, 2008).

Cytokinins show significant effects on seed germination under stress conditions. It helps to reduce the adverse effect of salinity, drought, heavy metal, and oxidative stress during germination (Peleg and Blumwald 2011; Miransari and Smith 2014). Cytokinins play a role during germination and cell division. It enhances the action of meristematic cells of epicotyls and hypocotyls (Heyl et al., 2012). Kinetin is a cytokinin that enhances plant growth and also involved in leaf expansion, senesence and plant development (Shah, 2007).

Auxins have no harmful effects on plants. It enables the formation of the roots of many plant species (Vanderhoef and Dute, 1981). Growth and development are important for the plant life cycle just as IBA is beneficial for the regulation of the plant life cycle. It plays a role in embryo development and elongation stages (Hauvermale et al., 2012) and in the expression of IBA levels by transcriptonal factors (Hayashi, 2012). It is reported that synthesis of auxin has occured in seed radicle tip during and after seed germination.

Younesi and Moradi (2015) reported that seed priming with GA_3 under salinty stress showed improved seed germination and seedling emergence of *Melissa officinalis* L. and *Cuminum cyminum* L. plants. Bhargava et al. (2015) indicated that germination speed accelerated with application of GA_3 priming in *Snapdragon* plants as compared to the control group. Karimi and Varyani (2016) pointed that the highest value of germination percentage was in priming with 100 mg/L GA_3 for *Calendula* seeds. In addition, catalase activity and total soluble sugars increased in primed seed with GA_3. Talebi et al. (2019) reported that the highest germination uniformity has been obtained with gibberellic acid priming for *Capparis spinosa* L. Zahedi et al. (2012) indicated that priming with GA_3 has positively affected seed germination, root and shoot length of Sweet William. Sneideris et al. (2015) reported that seed priming with GA_3 and IBA of pigeon pea under exposure to cadmium stress showed improved germination percentage. Rishu et al. (2018) indicated that high seedling emergence was obtained for pansy seeds (*Viola* × *wittrockiana* Gams.) as a result of priming with 20 mg/L GA_3. Mukherjee (2018) noticed that the highest germination percentage was realised in priming *Valeriana jatamansi* seeds with 200 ppm kinetin. In addition, it was observed that germination started earlier priming with 150 and 200 ppm IBA treatments as well as 200 and 250 ppm in kinetin when compared to other concentrations.

2.8.2 Cold stratification

Stratification is another type of pre-sowing treatment. It is a technique that helps to break seed dormancy. Cold stratification is used to mitigate the physiological dormancy of the seeds. Warm stratification is applied for morphological dormancy. On the other hand, a combination of cold and warm stratification is used for seeds having both morpho-physiological dormancy. The process of cold stratification is applied under cold and moist conditions with temperature between 0 and 10 °C for treatment depending upon the species (Baskin and Baskin, 2014) Peat, perlit, vermiculate and sand is prefered as substrate for cold stratification (Karlsson et al., 2014). The purpose of cold stratification is to improve the chilling requirement of seeds, help in the uptake of water for embryo which subsequently increase the germination of seed and results in robust seedling (Özçağıran 1979; Ercişli, 1992). Duration of cold stratification varies among different types of seeds (Baskin and Baskin, 2014). Thus, it ensures germination under optimum conditions (Probert, 2000). Numerous studies have reported the beneficial role of cold stratification for conferring biochemical and structural changes in seeds (Bewley et al., 2013). Study on *Verbascum calycosum* has demostrated that cold stratification has increased germination rate as compared to the control group (Hilooğlu et al., 2018). Cold stratification is known to promote the development of somatic embryo for holm oak plants (Mauri and Manzanera, 2004). It is also reported that cold stratification increased germination percentage of seeds of drought tolerant plants. *Cercis siliquastrum* L. is a wild plant propagated by seeds for ornamental purposes and seeds have dormancy. Study on this specie indicated that the efficient method to break dormancy and increase seed germination is cold stratification (Pipinis et al., 2011). *Magnolia grandiflora* L. is prefered for landscaping and has been observed to have germination parameters positively affected by different intervals of cold stratification treatments (Fetouh and Hassan, 2014). It is known that different amounts of water applied in cold stratification enhanced the breaking of dormancy in some weed species (Hu et al., 2018). It is reported that germination capacity of seeds of wild musk populations increased after cold stratification treatment (Aiello et al., 2017). It has been reported that cold stratification has increased some biochemical metabolites and decreased some of them depending on germination of *Glehnia littoralis* seeds with dormancy (Shao et al., 2020).

2.9 Characterization of Plant Genetic Diversity using Molecular Markers

Genetic diversity is one of the conservation strategies for the sustainability of biological diversity. Genetic variation contributes to the increase in biodiverstiy (Hussain and Nisar, 2020). It is as a result of evolution which causes changes in gene flow and mating systems in intra-species and inter-species (Nadeem et al., 2018). Knowledge about the genetic background of plants is important (Cole, 2003). Adaptation to abiotic and biotic stress factors are based on genetic diversity. Genes and gene products are responsible for the adaptation of species to the protection of populations against harmful effects of changes in ecological conditions (Hussain and Nisar, 2020). Genetic diversity is a very important source of variation that helps plant breeders in developing new cultivars with improved traits. These situations emphasised the importance of plant genetic resources that provide genetic diversity of plant species and secure food supply in future. Conservation strategies for plant genetic resources are categorized in three stages, the maintenance, characterization and evolution stage. Information on genetic variation of accessions are obtained by characterization (Laurentin, 2009).

Identification of genetic variation of plant population provides some analyses using morphological, biochemical and molecular marker techniques (Hussain and Nisar, 2020). Morphological markers are a technique based on measuring phenotypic and argonomic characteristics (Eagles et al., 2001). Morphological markers require field trials to evaluate plant characteristics. This is time-consuming and labor intensive. Therefore, studies on morphological marker leads to high cost. In addition to it is majority affected by environmental changes affecting plant life cycle (Eagles et al., 2001; Hussain and Nisar, 2020).

Isozymes, allozymes and storage proteins are used as biochemical markers (Hussain and Nisar, 2020). Allozymes is product of structural genes and gene duplications. It is the cheapest and easiest marker system used to obtain gene and genotype frequency (Berta et al., 2015). It is a co-dominant marker system as well. Genetic diversity, gene flow has been well investigated using biochemical markers (Mateu-Andres and De Paco, 2005). There are some disadvantages of biochemical markers; having less number of isozymes as markers, represented small portion in genome (Mondini et al., 2009).

Molecular markers are known as DNA markers that comprise of nucleotide sequences (Laurentin, 2009; Mondini et al., 2009; Sica et al., 2005). Molecular markers are the most reliable among marker techniques to determine polymorphisms of accessions (Hussain and Nisar, 2020). They have dominant and co-dominant (inheritance) features (Sica et al., 2005). Data obtained by molecular markers give valuable and clear information about population, alleles and diversity. These opportunities are important for breeders as they manage how to include plant genetic resources in breeding programmes for improvement of cultivars (Govindaraj et al., 2015). Molecular markers can be exploited in different ways, for mapping studies, heredity tests and marker assisted selection studies (Idrees and Irshad, 2014).

Use of molecular markers started in the late 20th century, their use broadened because of their vast applicability for improving plant characteristics. Plant breeders can enhance quality traits of plants in less time using molecular markers as compared to conventional plant breeding techniques (Idrees and Irshad, 2014). Molecular markers are also preferred for investigation in taxonomic studies (Hussain and Nisar, 2020).

Ideal molecular markers have properties such as high polymorphism, uniform distribution in genome and easy performance. Molecular markers are easy to use, simpler, quicker and cheaper as compared to other markers and their products are reproducible and reliable. The pleitropy is also not common in these marker type. The demand on low quantity of DNA also makes this marker type very advantageous (Hussain and Nisar, 2020).

Classification of molecular markers was based on the following concepts; mode of expression of alleles such as co-dominant or dominant markers, based on generation techniques such as hybridization-based or polymerase chain reaction (PCR)-based markers and way of inheritance either paternal or maternal inheritance and both as well (Nadeem et al., 2018).

Many molecular markers have been explored and widely used in plant sciences. Those molecular markers and their properties are briefly listed in Table 2.1 (Nadeem et al., 2018).

Table 2.1. Comparison of important characteristic of the most commonly used molecular markers

Characteristic	RLFP	RAPD	ALFP	ISSR	SSR	SNP	DaRT	Retrotransposons
Co-dominant/ Dominant	Co-dominant	Dominant	Dominant	Dominant	Co- dominant	Co- dominant	Dominant	Dominant
Reproducubility	High	High	Intermediate	Medium-high	High	High	High	High
Polymorphism level	Medium	Very high	High	High	High	High	High	High
Required DNA quality	High	High	High	Low	Low	High	High	High
Required DNA quantity	High	Medium	Low	Low	Low	Low	Low	Low
Marker index	Low	High	Medium	Medium	Medium	High	High	High
Genome abudance	High	Very high	Very high	Medium	Medium	Very high	Very high	High
Cost	High	Less	High	High	High	Variable	Cheapest	Cheapest
Sequencing	Yes	No	No	No	Yes	Yes	Yes	No
Status	Past	Past	Past	Present	Present	Present	Present	Present
PCR requirement	No	Yes	Yes	Yes	Yes	Yes	No	Yes
Visualization	Radioactive	Agarose gel	Agarose gel	Agarose gel	Agarose gel	SNP-VISTA	Microarray	Agarose gel
Required DNA (ng)	10000	20	50	50	50	50	50-100	25-50

2.9.1 Assesment of plant diversity by inter-Primer Binding Side (iPBS) marker

A part of the genome of eukaryotic organisms consists of repetitive DNA sequences known as transposable elements (TEs), which are of two types; class I and II. Class I and II referred to retrotranposons (RTs) and DNA transposons, respectively (Kalendar, 2011). Retrotranspons are in abundance than DNA transposons (Lander et al., 2001; Alzohairy et al., 2013). RNA intermediate enables the transfer of RTs in genomes and genome sizes increase due to their replication. Increase of total DNA in higher plants occur by RTs (Kalendar, 2011). RTs are divided into two groups, the long terminal repeats (LTRs) retrotransposons and non-LTR-retrotransposons (non-LTR-RTs). LTR-retrotransposons contain two subgroups; *Copia* and *Gypsy*. *Copia* refers to high copy number and *gyps* refers to high transposing activity (Xiong and Eickbush, 1990; Havecker et al., 2004; Jurka et al., 2007). LTR-RTs are more abundant than non-LTR-RTs (Arabidopsis Genome Initiative 2000; Rice Chromosome 10 Sequencing Consortium 2003). Retrotransposons have the ability to be used as molecular markers for genomic diversity due to their specific properties such as conserved pattern in structure and motif and high copy number (Kalendar, 2011). A single locus/inserted next to or within each other (nested RTs) is three specific arrangements of RTs. This pattern increases the polymorphism within and among species (Alzohairy et al., 2014). Nucleotide sequences inserted into the genome can be easily determined by retrotransposon marker systems (Kalendar, 2011). RTs molecular marker techniques are performed by PCR and they have the ability to identify a large part of the genome compared to other marker methods (Alzohairy et al., 2014). Generally they are preferred to use due to the ease, simplicity, applicability, and genotype resolution. It has been commonly used for genetic diversity studies and marker-assisted selection (Kalendar, 2011). It is known that sequence information is necessary to generate primers for RTs molecular marker approaches. This limitation has been resolved by iPBS (Inter-primer binding sequence**)** marker systems. This method is used to demostrate polymorphism using retrotransposons (Alzohairy et al., 2014; Kalendar, 2011).

A portion of Retrotransposon's internal domains contain primer binding sequence (PBS) (Alzohairy et al., 2014). Occurrence of iPBS amplification depends on a complementarity with tRNA on a reverse transcriptase primer binding site (PBS) in a LTR retrotransposon site. The isolation procedure is different from that in other

retrotransposons techniques. Besides in endogenous retroviruses, this isolation procedure is suitable for *Gypsy* and *Copia* LTR retrotransposons and nonautonomous LARD and TRIM elements from plants to animals. Therefore, the iPBS technique has enabled scientists to get strong DNA fingerprinting (Kalendar, 2011). Although sequence informations are needed to produce new primers as mention above, isolation of LTR retrotransposons have been successful in approximately all organisms as they are considered universal marker systems. iPBS primers are used via retroviruses, LTR retrotransposons and their cellular tRNAs. tRNA attaches to the primer binding site (PBS) near to the 5'-LTR region and begins to synthesize cDNA (Kalendar, 2011). In addition, another LTR retrotransposons can be used to prime cDNA synthesis instead of tRNA primer in vertebrates, maize and fungi (Kalendar, 2011; Hizi et al., 2008).

Recently, many studies have been conducted using iPBS markers to evaluate the genetic diverstiy in plants sciences. Doungous et al. (2020) reported that iPBS primers showed polymorphic rate in African *Gnetum* species which is classified as endangered. Yildiz et al. (2020) indicated that iPBS are reliable markers for breeding programmes as a result of its use in assessing the genetic diverstiy and population structure of Turkish pepper germplasm. Demirel et al. (2018) has used iPBS markers to reveal genetic similarity of potato genotypes and to develop new cultivars having desirable features.

CHAPTER III

MATERIALS AND METHODS

This thesis study was intended to investigate and reveal the morphological and physiological traits of two wild ornamental plant species collected from the Nigde region by applying different pre-sowing seed treatments on them and molecularly characterizing them to show their phylogenetic relationship. The materials and methods employed in this thesis are explained in detail subsections.

3.1 Selection of Plant Species

This thesis is aimed at selecting species of ornamental plants that can be used in landscape architecture. Wild plant species that have ornamental value were investigated at NOHU Campus area in coordination with Aladağ mountain from 2016 to 2018. *C. orientalis* and *C. raveyi* were identified to have ornamental values therefore, they were selected for morphological, physiological and molecular investigation. All experiments were conducted in the laboratories and greenhouse of the Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University. Investigation of plant species began during early spring of consecutive years at Niğde Ömer Halisdemir University campus area. Details of the selected plant species are given in subsection.

3.1.1 *Consolida orientalis* (Gay) schröd. (Ran)

The plant was observed between May and June when it was at its flowering stage on the NOHU campus (Figure 3.1). The specie was collected at flowering stage and sampled as a herbarium material. In addition, the life span of the specie was periodically observed over the years of study.

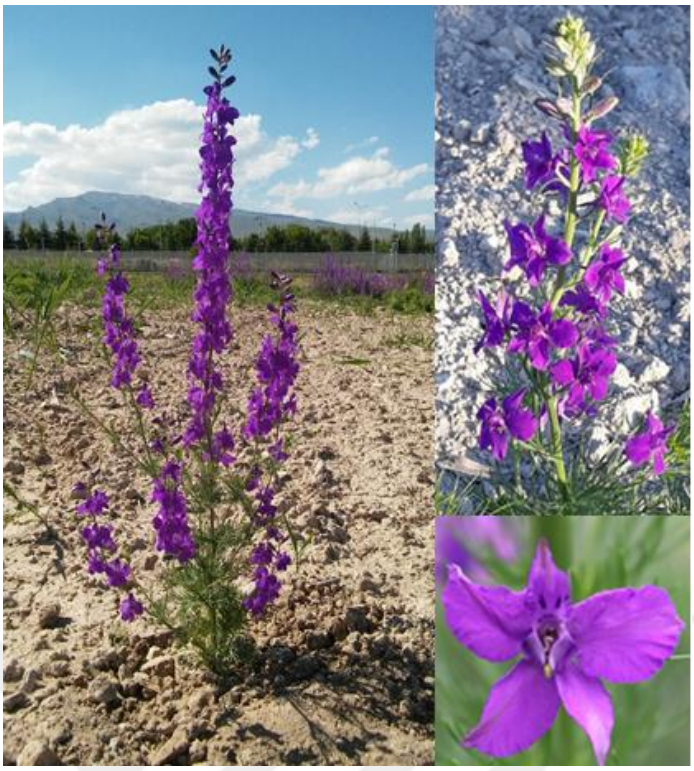

Figure 3.1. *Consolida orientalis* (Gay) Schröd. (Ran)

3.1.2 *Consolida raveyi* (Boiss.) Schrödinger

The plant was observed between June and July at flowering stage at NOHU campus area (Figure 3.2). The specie was collected at flowering stage and sampled as a herbarium material. In addition, the life span of the specie was periodically observed over the years of study.

Figure 3.2. *Consolida raveyi* (Boiss.) Schrödinger

3. 2 Collection of Seeds as Propagation Materials

Mature seeds were collected from the species after the flowering stage. Seeds of *C. orientalis* and *C. raveyi* were collected at different times as fruits opened between June and July as well as July and August of the consecutive years (Figure 3.3). The harvested plants were dried at room temperature for a week separating mature seeds from their capsule and stored in paper bags at room temperature and 4°C for six months.

Figure 3 3. Seeds of *C. orientalis* (a) and *C. raveyi* (b)

3.3 Effect of Pre-sowing Seed Treatments on Seed Germination

The effects of different pre-sowing seed treatments on seed germination in *C. orientalis* and *C. raveyi* were investigated under *in vitro* and *in vivo* conditions. These pre-sowing seed treatments included cold stratification (at 4ºC for 15, 30 and 45 days), PGR priming, with three different PGRs, GA_3, IBA, kinetin, at seven different concentrations, 5, 15, 30, 50 100, 300, 500 ppm and under three different temperatures, 10ºC, 15ºC, 20ºC.

3.3.1 Cold stratification

The aim of this experiment was to determine the effect of cold stratification and durations of seed germination of the two species under laboratory condition. Peat was used as stratification media and the seeds that were stored at room temperature were used for the cold stratification. Seeds were surface sterilized in 70% ethyl alcohol (EtOH) for 2 min, in 20% sodium hypochlorite solution for 10 mins then rinsed with sterile distilled water. Seeds were then placed into plastic Petri plates of diameter 11 cm to contain the propotion of peat and kept at 4°C over the period of either 15, 30 or 45 days for cold stratification (Figure 3.4). Humidity control was carried out after every 5-days, distilled water was given depending on the requirement. The experiments were carried out in five replications with 30 seeds in each replication. After durations were completed, Petri dishes were placed in a germination cabinet at an adjusted temperature of 20°C and dark period. Nonstratifed seeds were used as a control group. Seeds were considered to be germinated when the radicle began to elongate from the surface of peat. The number of germinated seeds was recorded daily for 21 days. Germination percentage was calculated at 21st day of germination.

Figure 3.4. Cold stratification at 4 °C

3.3.2 Seed priming with plant growth regulators (PGRs)

The reason for seed priming with PGRs at different concentration was to determine their effects on seed germination under three different temperature regimes of laboratory conditions. The PGR priming was applied on *C. orientalis* and *C. raveyi* seeds which were stored under two separate conditions, seeds stored at 4°C and those stored at room temperature.

3.3.2.1 Seed priming agents

GA_3, kinetin and IBA were used as priming agents at seven different concentrations for the germination experiments. Details of priming media, their concentrations and storage conditions are listed in Table 3.1 and Table 3.2.

Table 3.1. PGRs concentrations and seed storage at room condition

PGRs	Concentration (ppm)							Storage Condition
GA_3	5	15	30	50	100	300	500	
Kinetin	5	15	30	50	100	300	500	Room temperature
IBA	5	15	30	50	100	300	500	

Table 3.2. PGRs concentration and seed storage at 4 °C

PGRs	Concentration (ppm)							Storage Condition
GA_3	5	15	30	50	100	300	500	
Kinetin	5	15	30	50	100	300	500	4 °C
IBA	5	15	30	50	100	300	500	

3.3.2.2 Seed sterilization and PGRs priming

Before priming, seeds of plant species were surface sterilized in 70% EtOH for 2 min and 20% sodium hypochlorite solution for 25 min (including 2 drops of Tween 20) to prevent contamination. After that seeds were rinsed with sterile distilled water. Different concentrations of PGRs were prepared according to Table 3.1 and Table 3.2 and sterilized with syringe filter under sterile conditions. The sterilized seeds were incubated with PGRs solutions for 24 h and shaken at 100 rpm for efficent diffusion. Seeds were taken out from PGR solution after 24 h incubation, rinsed with sterile distilled water thrice. After the incubation period, seeds were dried under sterile cabinet at room temperature overnight.

3.3.2.3 Seed sowing and germination experiments

Dried seeds were sown in half-strength MS0 media containing 1.1 g/L MS salt (Murashige ve Skoog, 1962), 5 g/L sucrose (Duchefa) and 7 g/L phytoagar (Duchefa) plastic sterile containers (Duchefa). The pH of the medium was adjusted to 5.7 or 5.8. Germination experiments were conducted in the Laboratories of the Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University.Seeds were grown in a germination cabinet and the temperatures adjusted to 10°C, 15°C and 20°C

to observe the effects of storage conditions and seed priming on germination under dark conditions. Experiments were performed in 5 replications with 20 seeds in each replication. Dry seeds which were stored at room temperature and 4°C were used as control group (non primed). Seeds were considered germinated when radicle protrudes to about 1 mm. Seed germination were recorded daily for 21 days and percentages calculated at the 21st day of germination.

3.3.3 Germination percentage (%)

Germination percentage (%) was calculated by following the formula (ISTA, 1996) described below.

GP=(Number of normally germinated seeds / total number of seeds) × 100

3.4 Determination of Morphological Characteristics of Selected Wild Plant Species for Landscape Arthitecture under Greenhouse Condition

3.4.1 Effect of cold stratification on seed germination percentage under semi controlled greenhouse

Seeds were cold strafied for 45 days and germinated for further studies. Procedure of cold stratification experiment is as mentioned in section 3.3.1. Later, the Petri plates were shifted to the greenhouse of Plant Production and Technologies Department of NOHU on 15th March 2018 and 15th March 2019. Greenhouse temperature was adjusted to 25^{o}C/18^{o}C. The moisture status of Petri plates was checked every day, germination recorded daily for 21 days and germination percentage calculated according to the formula given in section 3.3.3.

Germinated seeds were taken from the Petri plates and transferred into plastic viols containing a mix of peat and perlite (4:1) to obtain healthy seedlings (Figure 3.5, Figure 3.6). When plantlets matured to 5-10 cm, plantlets were transferred into pots (2 L) containing a mix of peat and perlite (4:1), (Figure 3.4). Experiments were conducted in 3 replications with 10 pots for each species. Morphological data were collected at fully flowered stage of plant species.

Figure 3.5. Seedlings of *C. orientalis*

Figure 3.6. Seedlings of *C. raveyi*

Figure 3.7. Plantlets of *C. orientalis* (a) and *C. raveyi* (b)

3.4.2 Morphological measurements

Morphological measurements mentioned below were conducted during the fully flowered stage of the plant species (Figure 3.8, Figure 3.9).

Figure 3.8. An example of a fully flowered *C. orientalis*

Figure 3.9. An example of a fully flowered *C. raveyi*

3.4.2.1 Plant height

Plant height was measured by using measuring tape/cm from base to tip of plants.

3.4.2.2Stem diameter

Stem diameter of plant was measured by using digital vernier caliper/mm. Stem diameter was taken 10 cm above from base of stem. Data was taken in three replicates from each plant.

3.4.2.3 Main inflorescence length

Main inflorescence length was measured by using measuring tape/cm from edge of stem to tip of plants. Data was taken in three replicates from each plant.

3.4.2.4 Internode lenght in main infloresence

Internodes length of main infloresence was measured by using digital vernier caliber/mm from base, middle and top of main infloresence. Data was taken 5 times from each pot in three replicates.

3.4.2.5 Pedicel lenght in main infloresence

Pedicel length of main infloresence was measured by using digital vernier caliber/mm from base, middle and top of main infloresence. Data was taken 5 times from each pot in three replicates.

3.4.2.6 Number of flower in main infloresence

Number of flowers in main infloresence were counted for plants. Data was taken in three replicates from each plant.

3.4.2.7 Number of secondary inflorescences

Number of secondary inflorescences were counted for plants. Data was taken in three replicates from each plant.

3.4.2.8 Secondary branch length

Secondary branch length which were positioned in lower, middle and upper part of stem was measured with by using measuring tape/cm. Data was taken in three replicates from each plant.

3.4.2.9 Secondary inflorescence length

Secondary infloresence length was measured by using measuring tape/cm which was used for measurement of secondary branch length. Data was taken in three replicates from each plant.

3.4.2.10 Number of flowers in secondary infloresence

Number of flowers in **s**econdary infloresence was counted which was used for measurement of secondary branch length. Data was taken in three replicates from each plant.

3.4.2.11 Internode lenght in secondary infloresence

Internodes length of secondary infloresence was measured by using digital vernier caliber/mm which were used for measurement of secondary branch length. Data was taken 5 times from each pot in three replicates.

3.4.2.12 Pedicel lenght in secondary infloresence

Pedicel length of secondary infloresence was measured by using digital vernier caliber/mm which were used for measurement of secondary branch length. Data was taken 5 times from each pot in three replicates.

3.4.2.13 Flower lenght

Flower lenght of main and secondary infloresence was measured by using digital vernier caliber/mm Data was taken 5 times from each pot in three replicates.

3.4.2.14 Flower diameter

Flower diameter of main and secondary infloresence was measured by using digital vernier caliber/mm. Data was taken 5 times from each pot in three replicates.

3.5 Genetic Diversity of Ranunculaceae Species by iPBS molecular markers

Plant species belonging to the Ranunculaceae family were investigated at NOHU and some parts of the Nigde district. Plant species belonging to the genus *Consolida, Ranunculus, Nigella, Adonis* and *Delphinium* genus were found and sampled as herbarium materials. Fresh leaves of plants were stored at -80°C for molecular studies. Scientific names of plant species and sampling locations are listed in Table 3.3 below.

Table 3.3. Plant species and sampling location

Species name	Sampling location
Consolida orientalis	Campus area, NOHU
Consolida raveyi	Campus area, NOHU
Consolida hellespontica	Campus area, NOHU
Consolida thirkeana	Campus area, NOHU
Consolida regalis subsp. *paniculata var. Paniculata*	Campus area, NOHU
Ranunculus kotschyi	Kayaardı Bağları, Nigde
Ranunculus repens	Campus area, NOHU
Ranunculus cuneatus	Hançerli, Nigde
Adonis aestivalis subsp. *aestivalis*	Campus area, NOHU
Nigella arvensis subsp. *glacua*	Campus area, NOHU
Delphinium venulosum	Campus area, NOHU

3.5.1 Genomic DNA extraction

Fresh leaf tissues from each sample which were stored at –80°C were used for genomic DNA extraction. Genomic DNA was extracted from leaves of species using the standard CTAB protocol modified by Doyle (1991). The preparation of CTAB extraction buffer and total genomic DNA extraction method is given in detail below.

3.5.1.1 Chemicals and their preparation for CTAB extraction buffer

The concentration of chemicals needed to prepare CTAB extraction buffer of 500 mL were 50 mL 1 M Tris pH 8.0, 20 mL 0.5 M EDTA pH 8.0, 175 mL 4 M NaCl, 10 g CTAB, 10 g PVP 40 and 500 mg $Na_2S_2O_5$.

1 M Tris pH 8.0 (1 L): 121.1 g of Trizma base was dissolved into 800 mL of dH_2O. HCl was use to adjust the pH to 8.0 and the solution autoclaved after adding dH_2O to balance the volume.

0.5 M EDTA pH 8.0 (1 L): 186.1 g of EDTA was dissolved into 800 mL of dH_2O. 10 M NaOH was used to adjust the pH to 8. And before autoclaving, the volume of solution was made 1 L with dH_2O.

4 M NaCl: 243.3 g of NaCl was dissolved into 800 mL of dH_2O. Volume of solution was made 1 L with dH_2O.

Total genomic DNA extraction method

- Approximately 0.2 g of leaf tissues were grinded to powder using liquid nitrogen.
- 900 µL of CTAB extraction buffer was immediately added to the grinded leaves and transferred into 2 mL Eppendorf tubes.
- After adding the buffer, samples were vortexed to thoroughly mix the solution.
- Before starting the DNA extraction, the heat block was adjusted to 65°C to incubate the extracted samples for 1 h while using the vortex to mix each sample for between 5-10 seconds per minutes.
- Samples were taken from heat block after incubation and kept for 5 minutes at room temperature.
- Each sample, was hand shaken for 15 minutes after adding 900 µL of Chloroform-isoamyl alcohol mix (24:1).
- For another 15 minutes, the samples were centrifuged at 14.000 rpm.
- Approximately 700 µL of supernatants of the samples were carefully collected into new 1.5 mL Eppendorf tubes.
- Each sample in the new 1.5 mL Eppendorf tubes received 500 µL of 100% cold Isopropanol and was thoroughly mixed by hand oscillation for 15 minutes.
- The mixed samples were then centrifuged at 10.000 rpm to for 5 seconds. After which, the liquid phase was discarded.
- 1 mL of 76% Ethanol containing 10 mM of NaAc was later added to the tubes containing the samples and shaken for another 15 minutes.
- Pellets precipitated after 5 seconds of centrifugation at 7.000 rpm and then the liquid phase was discarded.
- After centrifugation, Eppendorf tubes containing the pellets were kept open at room temperture for the pellets to dry.
- The dried DNA samples were suspended in 100 µL distilled water and stored at -20 ^{o}C.

The quality and concentration of th extracted DNA was checked using a Shimadzu Biospec-nano UV–vis Spectrophotometer. Concentrations of the extracted DNA are recorded in Table 3.4. The extracted genomic DNA was diluted to 50 ng and loaded in wells which were prepared on 1% agarose gel to check the quality of the DNA. 1 kb DNA ladder (Thermo Scientific) was loaded to the first well of gel serving as a marker. Agarose gels were stained using ethidium bromide and bands visualized under Gel Doc™ XR+ gel imaging system (Bio-Rad). A gel picture of the extracted DNA is displayed in Figure 3.6.

Table 3.4. Concentration of extracted genomic DNA of plant species

Serial No	Species name	260/280	Concentration (ng/ul)
1	*Ranunculus kotschyi*	1.991	3101.75
2	*Ranunculus repens*	2.15	805.35
3	*Ranunculus cuneatus*	2.137	310.15
4	*Adonis aestivalis* subsp. *aestivalis*	2.008	2158.95
5	*Delphinium venulosum*	2.012	3605.1
6	*Consolida hellespontica*	2.19	1802.3
7	*Consolida regalis* subsp. *paniculata* var. *paniculata*	2.103	646.75
8	*Consolida thirkeana*	2.001	1404.3
9	*Nigella arvensisi* subsp. *glauca*	2.058	1826.85
10	*Consolida orientalis*	2.145	1034.9
11	*Consolida raveyi*	2.109	1200.35

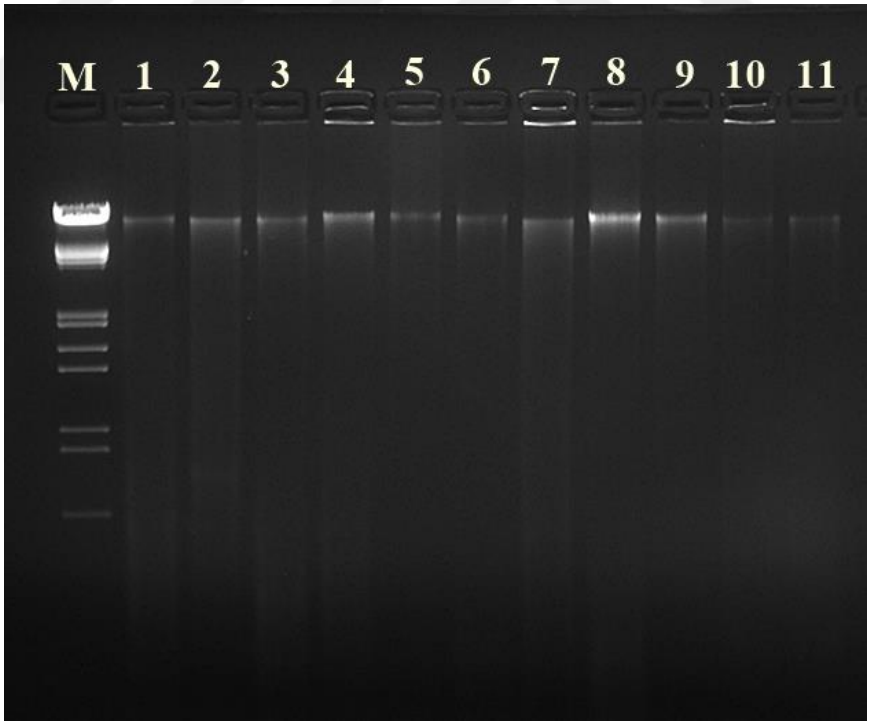

Figure 3.1. Gel picture of extracted DNA of plant species

3.5.1.2 iPBS (inter-PBS retrotransposon markers) and PCR conditions

iPBS retrotransposon markers developed by Kalendar et al. (2010) were used for the investigation of plant species under study for molecular characterization. Twenty of these primers were used for fingerprinting. Selected primers are given in Table 3.5, PCR conditions in Table 3.6, Table 3.7 and Table 3.8. After PCR was performed, the PCR products were analyzed on 1.8% agarose gel using 1× TAE buffer at 90 V for 2 h. To identify band size on the gel, a DNA ladder, GeneRuler DNA Ladder Mix (Thermo Scientific), was loaded into the first and the last wells on the gel. Agarose gels were stained using ethidium bromide and bands were visualized under Gel Doc™ XR+gel imaging system (Bio-Rad). Sharp and clear bands were scored after all PCR and electrophoresis analysis were completed. Polymorphism information content (PIC) was determined according to formula given below (Hinze et al., 2015).

$$PIC_b = 1-(p^2+q^2)$$

(*p* represents the frequency of the band prensence and *q* the frequency of the band absence of the *b*th band of the iPBS primer and PIC*b* is the PIC of the band b).

Table 3.5. Selected 20 iPBS primers, sequences and annealing temperature (Ta)

Name	Sequence	Ta (°C)
2074	GCTCTGATACCA	50
2075	CTCATGATGCCA	50
2095	GCTCGGATACCA	53
2229	CGACCTGTTCTGATACCA	52
2232	AGAGAGGCTCGGATACCA	55
2239	ACCTAGGCTCGGATGCCA	55
2252	TCATGGCTCATGATACCA	52
2272	GGCTCAGATGCCA	55
2274	ATGGTGGGCGCCA	63
2374	CCCAGCAAACCA	53
2375	TCGCATCAACCA	52
2377	ACGAAGGGACCA	53
2380	CAACCTGATCCA	50
2387	GCGCAATACCCA	52
2391	ATCTGTCAGCCA	52
2392	TAGATGGTGCCA	52
2399	AAACTGGCAACGGCGCCA	52
2400	CCCCTCCTTCTAGCGCCA	50
2402	TCTAAGCTCTTGATACCA	50
2237	CCCCTACCTGGCGTGCCA	55

Table 3.6. PCR components for 12-13 base pair (bp) of iPBS primer

Contents	Volumes	Final concentrations
DNA (5 ng/µL)	5 µL	25 ng
10X PCR buffer (with $MgCl_2$)	2.5 µL	1X
dNTPs (10 mM)	0.375 µL	200 µM
iPBS primer (12-13 bp)	3 µL	1 µM
Dream Tag polymerase (5U/µL)	0.2 µL	1.00 U
dH_2O	11.925 µL	
Total	25 µL	

Table 3.7. PCR components for 18 base pair (bp) of iPBS primer

Contents	Volumes	Final concentrations
DNA (5 ng/µL)	5 µL	25 ng
10X PCR buffer (with $MgCl_2$)	2.5 µL	1X
dNTPs (10 mM)	0.375 µL	200 µM
iPBS primer (12-13 bp)	5 µL	1 µM
Dream Tag polymerase (5U/µL)	0.2 µL	1.00 U
dH_2O	9.925 µL	
Total	25 µL	

Table 3.8. PCR amplification conditions

Steps	Temperature	Duration	Cycles
1.First denaturation	95 °C	3 minute	1
2.Denaturation	95 °C	15 second	
3.Annealing	* °C	60 second	35
4.Extension	72 °C	2 minute	
5.Final extension	72 °C	7 minute	1

*Annealing temperature of primers given in Table 3.5

3.6 Statistical Analysis

All statistical analyses were conducted using the procedures of SAS and NTSYS programs (SAS, 1990; Rohlf, 1997). The descriptive statistics were carried out using the TABULATE procedure of SAS and among them only means were presented (SAS, 1990). All ANOVAs were consturcted using the GLM procedure employing the factorial or split-plot design based on the experimental design used (SAS, 1990).

The iPBS data were recorded as 1 for the presence of a band and 0 for its absence. The data was used to consturct a similarty index file using NTSYS program (Rohlf, 1997). They were also subjected to a multivaraite analysis, Principle Coordinate (PCoA) generating a three dimensinal scattered plot and dendrograms using the UPGMA (Unweighted Pair Group Method using Arithmetic Average) (Rohlf, 1997).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Seed Germination Studies and Pre-Sowing Seed Treatments

4.1.1 Cold stratification

Analysis of variance (ANOVA) results are presented in Table 4.1 showing the effect of cold stratification on germination percentage for both species. According to results, species, duration and interaction between species and duration was found to be significant regarding germination percentage.

Table 4.1. ANOVA table showing germination percentage of species after cold stratification

Source	Degree of freedom	Mean Square
Species (S)	1	5601.1*
Replication (R) / S	8	18.5
Duration (D)	1	5393.0*
S x D	8	2030.0*
Error	1	79.8

*indicates significance at $p \leq 0.05$.

According to the results shown in Table 4.2, cold stratification positively affected seed germination for both species. *C. raveyi* seeds had higher germination percentage (50.3%) than that of *C. orientalis* seeds (26.7%). Cold stratification durations for 15 days and 30 days improved seed germination as compared to those stratified for 45 days and those in the control group. On the other hand, species and stratification duration were found to be significant with respect to germination percentage. *C. orientalis* seeds that were stratified for 45 days showed increase in seed germination. The most effective duration for germination percentage was found to be cold stratification-treatment of 15 days for *C. raveyi* seeds and 45 days for *C. orientalis*.

Table 4.2. Mean value showing effect of cold startification and durations on germination percentage for both species

Source		Mean
Species		
C. orientalis		26.7b
C. raveyi		50.3a
Duration		
Control		5.3c
15 d		56.0a
30 d		53.0a
45 d		39.7b
Interaction		
C. orientalis	Control	0.0d
	15 d	26.7c
	30 d	36.7b
	45 d	43.3a
C. raveyi	Control	10.7d
	15 d	85.3a
	30 d	69.3b
	45 d	36.0c
Overall		38.5

Different letters indicate significant differences in by Tukey test at 0.05.

Selection of appropriate pre-treatment methods are necessary for obtaining good seed germination rates (Seng and Cheong, 2020). Many studies reported that cold stratification is a pre-treatment to break dormancy and improve seed germination (Kosa and Karagüzel 2020,; Chen et al., 2015; Zhou et al., 2009). Aiello et al. (2017) reported that 14 days of cold stratification increased germination percentage of wild musk yarrow seeds. Tang et al. (2019) indicated that higher germination percentage was obtained when seeds of *Sorbus alnifolia* that have high ornamental potential were cold stratified for 150 days. On the other hand, Luo et al. (2020) pointed out that durations of cold stratification were important to the rise in seed germination. Previous and current studies suggests that cold stratification is an effective way to enhance seed germination.

4.1.2 Seed priming with PGR

The PGR priming was conducted on *C. orientalis* and *C. raveyi* seeds and their effects was observed on germination percentage.

4.1.2.1 Effect of seed priming using GA_3 on *C. orientalis* seeds

4.1.2.1.1 Germination percentage (%)

Table 4.3 represents the results of an ANOVA indicating the effect of seed priming on the germination percentage of *C. orientalis* seeds treated with GA_3. According to the results, concentration, temperature and their interaction were found to be significant for both seed storage conditions.

Table 4.3. ANOVA table showing germination percentage of *C. orientalis* seeds after priming with GA_3

		4°C	Room
Source	**Degree of freedom**	**Mean Square**	**Mean Square**
Concentration (C)	7	621*	309*
Temperature (T)	2	37053*	28723*
C x T	14	288*	180*
Error	96	78	100

*indicates significance at $p \leq 0.05$.

Table 4.4 contains the mean values of germination percentage of *C. orientalis* seeds after priming with GA_3 under both seed storage conditions. According to the results, it was observed that the seeds treated with GA_3 at concentration of 300 ppm showed better results regarding germination percentage (35.7%) for seeds stored at 4°C. However, at other concentrations, there was a decline in the seed germination percentage as compared to 300 ppm GA_3. On the other hand, the maximum germination percentage was observed at 15 ppm GA_3 for seeds stored at room temperature but it was statistically same group with the control. However, the lowest germination result was detected treatment with 500 ppm GA_3. Temperature also has an effect on seed

germination; hence, different temperatures for seed germination were evaluated and compared to find a more favourable temperature condition for the growth of these seeds. Higher seed germination percentages were observed at 10°C under both storage conditions while, no seed germination was observed at 20°C (Table 4.4).

Table 4.4. Mean value showing effect of GA_3 at different temperature with different concentration on germination percentage of *C. orientalis*

	4 C°	**Room**
Source	**10°C-15°C-20°C**	**10°C-15°C-20°C**
GA_3 concentration (ppm)	**Mean**	**Mean**
Control	31.3ba	28.0a
5	18.3c	24.3ba
15	15.7c	31.3a
30	23.3bc	27.0ba
50	23.7bc	26.3ba
100	24.0bc	22.7ba
300	35.7a	23.3ba
500	24.7bc	16.0b
Temperature (°C)		
10	58.6a	53.3a
15	15.1b	21.4b
20	0.0c	0.0c
Overall	24.6	24.9

Different letters indicate significant differences in by Tukey test at 0.05.

4.1.3 Effect of seed priming using IBA on *C. orientalis* seeds

4.1.3.1 Germination percentage (%)

From the ANOVA table, the effect of PGR priming using IBA on the germination percentages of *C. orientalis* seeds that were stored at 4°C and under room temperature were observed. In the table 4.5, significant differences were observed in terms of concentration, temperature and interaction between concentration and temperature under both storage conditions.

Table 4.5. ANOVA table showing germination percentage of *C. orientalis* seeds after priming with IBA

		4°C	Room
Source	**Degree of freedom**	**Mean Square**	**Mean Square**
Concentration (C)	7	328*	156*
Temperature (T)	2	18154*	27638*
C x T	14	224*	61*
Error	96	50	68

*indicates significance at $p \leq 0.05$.

According to the results in Table 4.6, germination percentages of *C. orientalis* seeds that were stored at 4°C decreased after treatment with different concentrations of IBA as compared to those in the control group. Besides, application of 500 ppm IBA for seeds stored at room temperature increased germination percentage (30.3%) as compared to the control (28%). Contrarily, other concentration of IBA exhibited similar results like the control group. One of the main factor affecting seed germination is temperature. The results depicted different responses of seed to germination at different temperatures. It was higher at 10°C than 15°C for seeds that were kept at both 4°C and room temperature conditions. However, there was no germination observed at 20°C, in the IBA treatments just as was noticed in GA_3.

Table 4.6. Mean value showing effect of IBA at different temperature with different concentration on germination percentage of *C. orientalis* seeds

	4 C°	Room
Source	**10°C-15°C-20°C**	**10°C-15°C-20°C**
IBA concentration (ppm)	**Mean**	**Mean**
Control	31.3a	28.0ba
5	19.7b	26.3ba
15	16.7b	25.7ba
30	16.3b	22.7ba
50	19.0b	19.7ba
100	20.3b	25.0ba
300	19.7b	30.3a
500	20.0b	26.0ba
Temperature (°C)		
10	42.5a	52.5a
15	18.6b	23.9b
20	0.0c	0.0c
Overall	20.4	25.5

Different letters indicate significant differences in by Tukey test at 0.05.

4.1.4 Effect of seed priming using kinetin on *C. orientalis* seeds

4.1.4.1 Germination percentage (%)

The results of this study as recorded in the ANOVA table shows the effects of seed priming by using kinetin on germination percentage of *C. orientalis* seeds. According to the results, the concentration, temperature and their interaction were found to be significant for seeds stored at both 4°C and room temperature conditions.

Table 4.7. ANOVA table showing germination percentage of *C. orientalis* seeds after priming with kinetin

Source	Degree of freedom	4°C Mean Square	Room Mean Square
Concentration (C)	7	1327*	1537*
Temperature (T)	2	27698*	10601*
C x T	14	955*	1078*
Error	96	68	130

*indicates significance at $p \leq 0.05$.

A representation of the mean values of germination percentage of *C. orientalis* seeds after priming with kinetin under both storage conditions are in Table 4.8. The results indicated a reduction in germination percentage in all concentrations for seeds stored at 4°C. The maximum germination percentage was noted with application of 5 ppm kinetin (34%) and minimum seed germination after application of 100 ppm kinetin (5%) for seeds stored under room temperature conditions. Additionally, the results clearly indicate that temperature affected seed germination under both conditions. Germination percentage was found to be higher at 10°C as compared to 15°C. There was no germination obtained at 20°C.

Table 4.8. Mean value showing effect of kinetin at different temperature with different concentration on germination percentage of *C. orientalis* seeds

Source	4°C 10°C-15°C-20°C	Room 10°C-15°C-20°C
Kinetin concentration (ppm)	**Mean**	**Mean**
Control	31.3a	28.0ba
5	27.7ba	34.0a
15	30.3ba	23.0bac
30	18.3dc	16.3bdc
50	7.0e	10.7dc
100	9.7de	5.0d
300	28.3ba	8.0d
500	21.3bc	14.3dc
Temperature (°C)		
10	51.0a	32.3a
15	14.3b	20.0b
20	0.0c	0.0c
Overall	21.8	17.4

Different letters indicate significant differences in by Tukey test at 0.05.

4.1.5 Effect of seed priming using GA_3 on *C. ravei* seeds

4.1.5.1 Germination percentage (%)

The results in the ANOVA table shows the effects of seed priming using GA_3 on germination percentage of *C. raveyi* seeds. According to the results, concentration, temperature and their interactions were found to be significant for seeds stored at 4 °C. However, the ANOVA indicated that the seeds stored at room temperature did not show any change in their germination percentage after the application of GA_3 at different concentrations but the impact of temperature on germination was found to be significant.

Table 4.9. ANOVA table showing germination percentage of *C. raveyi* seeds after priming with GA_3

		4°C	Room
Source	**Degree of freedom**	**Mean Square**	**Mean Square**
Concentration (C)	7	175*	55
Temperature (T)	2	88610*	73141*
C x T	14	232*	97
Error	96	61	74

*indicates significance at $p \leq 0.05$.

Table 4.10 presents the mean values of germination percentage of *C. raveyi* seeds after PGR priming with GA_3 under both seed storage conditions. The findings indicated that application of 500 ppm GA_3 increased germination percentage of *C. raveyi* seeds (56.7%) as compared to those in the control group (49.3%). The germination percentage obtained under GA_3 concentration of 100 ppm and 300 ppm was similar to that in the control group. However, the use of different concentration of GA_3 did not affect germination percentage of the seeds stored at room temperature when compared to the control group. Also, the results obtained considering the effect of different temperatures on germination percentage showed that maximum germination percentage was observed at 10°C under both storage conditions. However minimum germination percentage was recorded at 20°C.

Table 4.10. Mean value showing effect of GA_3 at different temperature with different concentration on germination percentage of *C. raveyi* seeds

Source	4°C 10°C-15°C-20°C	Room 10°C-15°C-20°C
GA_3 concentration (ppm)	**Mean**	**Mean**
Control	49.3ba	54.0a
5	46.3b	52.0a
15	47.3b	51.0a
30	47.3b	52.7a
50	47.7b	55.0a
100	50.7ba	55.3a
300	52.0ba	56.3a
500	56.7a	55.7a
Temperature (°C)		
10	96.4a	90.9a
15	50.4b	64.0b
20	2.3c	7.1c
Overall	49.7	54.0

Different letters indicate significant differences in by Tukey test at 0.05.

4.1.6 Effect of seed priming using IBA on *C. ravei* seeds

4.1.6.1 Germination percentage (%)

The results of the ANOVA are documented in Table 4.11 showing the effects of seed priming using IBA on germination percentages of *C. raveyi* seeds. Significant differences were observed in the concentration, temperature and interaction between concentration and temperature under both conditions.

Table 4.11. ANOVA table showing germination percentage of *C. raveyi* seeds after priming using IBA

		4°C	Room
Source	**Degree of freedom**	**Mean Square**	**Mean Square**
Concentration (C)	7	454*	394*
Temperature (T)	2	77773*	71168*
C x T	14	328*	364*
Error	96	51	68

*indicates significance at $p \leq 0.05$.

Table 4.12 represents the mean values of germination percentage of *C. raveyi* seeds after priming with IBA. According to results, the highest germination percentage was observed at 100 ppm IBA while the least germination percentage was found at 30 ppm IBA treatment for seeds stored at 4 °C. Germination percentage was not statistically different for the control group, 100 ppm and 500 ppm IBA. Contrarily, the seeds stored at room temperature exhibited the highest germination percentage after priming with 300 ppm IBA. Whereas, minimum germination percentage was obtained after priming with 15 ppm IBA. In addition, temperature had significant effects on seed germination under both storage conditions. The results indicate that the highest germination percentage was obtained at 10°C as compare to 15°C and 20°C, for both storage conditions.

Table 4.12. Mean value showing effect of IBA at different temperature with different concentration on germination percentage of *C. raveyi* seeds

Source	4°C 10°C-15°C-20°C	Room 10°C-15°C-20°C
IBA Concentration (ppm)	**Mean**	**Mean**
Control	49.3a	54.0ba
5	39.3c	53.3ba
15	39.3c	43.3c
30	37.7c	43.7c
50	40.7bc	49.0bac
100	50.3a	49.3bac
300	47.7ba	58.0a
500	50.0a	47.3bc
Temperature (°C)		
10	90.4a	90.6a
15	40.0b	52.3b
20	2.5c	6.4c
Overall	44.3	49.8

Different letters indicate significant differences in by Tukey test at 0.05.

4.1.7 Effect of seed priming using kinetin on *C. ravei* seeds

4.1.7.1 Germination percentage (%)

The ANOVA table shows the effect of priming using kinetin on germination percentage of *C. raveyi* seeds stored at 4°C and under room temperature conditions. Table 4.13 shows that significant differences was noticed in concentration, temperature and interaction between concentration and temperature under both conditions.

Table 4.13. ANOVA table showing germination percentage of *C. raveyi* seeds after priming with kinetin

Source	Degree of freedom	4°C Mean Square	Room Mean Square
Concentration (C)	7	3592*	1772*
Temperature (T)	2	24718*	42758*
C x T	14	2823*	1316*
Error	96	91	84

*indicates significance at $p \leq 0.05$.

The maximum germination percentage was observed from the control group as compared to all concentrations for seeds stored at 4°C and under room temperature conditions, as well. Reduction in germination percentage was observed in all concentration of kinetic for seeds stored under both conditions. As shown in Table 4.14, temperature happens to be the most important factor affecting seed germination in this case. The germination percentage was found to be higher at 10°C as compare to 15°C and 20°C for both conditions.

Table 4.14. Mean value showing effect of kinetin at different temperature with different concentration on germination percentage of *C. raveyi* seeds

Source	4°C 10°C-15°C-20°C	Room 10°C-15°C-20°C
Kinetin concentration (ppm)	**Mean**	**Mean**
Control	49.3a	54.0a
5	39.7a	49.3ba
15	41.0a	53.0a
30	41.7a	48.3ba
50	28.7b	29.0d
100	12.7cd	25.7e
300	7.0d	36.7dc
500	19.0cb	40.0bc
Temperature (°C)		
10	50.8a	63.5a
15	36.5b	58.1b
20	2.4c	4.4c
Overall	29.9	42.0

Different letters indicate significant differences in by Tukey test at 0.05.

The influence of seed priming on seed germination was determined using different concentrations of GA_3, IBA and kinetin at different temperatures. The results indicated that maximum germination percentage was achieved by applying different concentration of GA_3, IBA and kinetin at 10 °C for both species. Therefore it was concluded that both species require low temperature for better seed germination because seed germination was negatively affected at higher temperatures. According to a study conducted by Serim and Sözeri (2011), germination percentage of *C. orientalis* seeds were found to be higher (31%) at 10°C for seeds stored at 4°C over a one month period, whereas seeds stored at room temperature for one month had 12% germination at 10 °C. According to their results, increased temperature had negative effects on seed germination of *C. orientalis* at 15°C and 20°C in both storage conditions. The optimum temperature was found to be 10°C for seed germination after different pre-treatments were carried out by Serim and Sözeri (2011). Our study also indicated that seed germination for both species was higher at 10°C and 15°C for both storage conditions

just as was in the case of Serim and Sözeri (2011) therefore this work confirms their findings. Whereas, there were no germinations observed in *C. orientalis* seeds at 20°C in both studies, seed germination was observed in *C. raveyi* at 20°C under both storage conditions in this current study. Saffari et al (2021) noted that high germination percentage was achieved in treatments with 100 ppm GA_3 in *Agrimonia eupatoria* L. uncoated seeds. Golmohammadzadeh et al. (2020) also reported that seed priming with plant growth regulators enhance breaking dormancy and seed germination. Enhancement of seed germination of *Papaver rhoeas* was observed after priming with 750 ppm GA_3 for 24 h. Additionally, seed germination of *Papaver dubium* was promoted using 500 ppm GA_3 for 48 h. Karimi and Varyani et al. (2016) demostrated that PGR priming with 100 ppm GA_3 for 72 h increased germination percentage on *Calendula officinalis* L. These findings agree with the results of our study confirming the role of PGR applied at specific concentrations and temperatures to produce desired seed germination. Kumari et al (2010) investigated the effect of priming with IBA on *Jatropha curcas* seeds with the highest germination percentage observed at 2 ppm IBA. The positive effect of priming with kinetin on seed germination of *Medicago truncatula* was declared by Araújo et al (2019).

4.2 Determination of Morphological Characteristics of Selected Wild Plant Species for Landscape Architecture under Greenhouse Condition.

4.2.1 Effect of cold stratification on seed germination percentage under semi controlled greenhouse

To detemine effect of cold stratification-treated 45 day was performed on *C. orientalis* and *C. raveyi* seeds. After durations were completed, Petri dishes were shifted under semi controlled greenhouse condition. Nonstratifed seeds was used as a control group. The number of germinated seeds were recorded daily until 21 days and germination percentage was determined for both species. The germinated seeds were removed from Petri dishes and planted in plastic viol to obtain seedlings for morphological investigation.

ANOVA results regarding the effect of cold stratification for 45 day on germination percentage of species are given in Table 4.15. The results indicated that duration and

interaction between species and duration were found to be significantly related with germination percentage.

Table 4.15. ANOVA table showing germination percentage of species after cold stratification under semi controlled greenhouse

Source	Degree of freedom	Mean Square
Year (Y)	1	2.5
Replication (R) / Y	8	26.4
Species (S)	1	122.5
R / Y,S	8	26.4
Duration (Day)	1	93122.5*
Y x D	1	2.5
S x D	1	122.5*
S x D x Y	1	2.5
Error	16	26.4

*indicates significance at $p \leq 0.05$.

The findings in Table 4.15 indicates that cold stratification for a 45 day period improved seed germination for both species. The highest germination percentage (97%) was obtained for *C. orientalis* seeds when compared to the control group (0%). On the other hand, *C. raveyi* seeds that were cold stratified for 45 days was promoted 100% increase in seed germination as compared to the control group.

Table 4.16. Effect of cold startification and duration on germination percentage of species under semi controlled greenhouse

Source		Mean
Species		
C. orientalis		47
C. raveyi		50
Year		
2018		49
2019		48
Duration		
Control		0b
45 day		97a
Interaction		
C. orientalis	Control	0
	45 d	93a
C. raveyi	Control	0
	45 d	100a
Overall		48

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2 Morphological Measurements

Following the vegetation period of plants species under semi controlled greenhouse conditions, *C. oreintalis* was observed to be at full flowered stage in May and *C. raveyi* reached full flowering stage in June during the study years.

4.2.2.1 Plant height

Plant height of different species were measured at fully flowered stage. The results of the ANOVA regarding plant height of species is given in Table 4.17. The results demonstrated that there were significant differences among species for plant height. On the other hand, there were no significant differences for plant height in respect to years and interaction between years and species.

Table 4.17. ANOVA table for plant height of species growing under semi controlled greenhouse condition

Source	Degree of freedom	Mean Square
Species (S)	1	16100.4*
Replication / P	4	11.3
Year (Y)	1	31.8
SxY	1	56.1
Error	4	10.2
Total	11	

*indicates significance at $p \leq 0.05$.

The average plant height of the different species grown under semi controlled greenhouse condition are given in Table 4.18. The highest plant height was founded in *C. orientalis* (122.7 cm) while the lowest plant height was measured for *C. raveyi* (49.59 cm). Karagüzel et al. (2006) reported that the plant height of *C. orientalis* grown in native ecological conditions were negatively affected by high temperature. This result indicated that temperature plays an important role in the growth of *C. orientalis*. Moreover, there was negative correlation between plant height and pH (≤ 8.3), potassium and calcium contents. On the other hand, altitude, organic matter, soil texture, EC, phosphorus and magnesium contents positively affected plant height. The average plant height measured were 67.3 cm (Cevizli) and 58.5 cm (Korkuteli) grown at different locations. Another study conducted in 2007 (Karagüzel et al., 2007), to determine the response of growth habits of *C. orientalis* at different sowing times under field and unheated greenhouse conditions. They found that there was interaction between growing conditions and sowing time on plant height. Plants which were grown under unheated greenhouse exhibited maximum plant height (134.5 cm) as compared to field condition (31.1 cm) in December. There were no statistical differences observed for plant heights for sowing times in September (124.2 cm) and Nowember (124.4 cm) under unheated greenhouse condition. These findings agree with the findings of this current study.

Table 4.18. Average values of plant height of species growing under semi controlled greenhouse condition

Source		**Mean**
Species		
C. orientalis		122.7a
C. raveyi		49.5b
Year		
2018		87.7
2019		84.5
Interaction		
C. orientalis	2018	122.2
C. raveyi	2018	53.3
C. orientalis	2019	123.3
C. orientalis	2019	45.7
Overall		86.1

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.2 Stem diameter

Stem diameter of the species under study were measured at fully flowered stage. The ANOVA regarding stem diameter is given in Table 4.19. The results demostrated that there were significant differences between species however, there were no significant differences observed for years and interaction between years and species.

Table 4.19. ANOVA table for stem length of different species growing under semi controlled greenhouse condition

Source	Degree of freedom	Mean Square
Species (S)	1	17.35*
Replication / R	4	0.04
Year (Y)	1	0.00
S x Y	1	0.10
Error	4	0.03

*indicates significance at $p \leq 0.05$.

The average value of stem diameter is given in Table 4.20. The highest stem diameter (5.6 mm) was measured in *C. orientalis* while the smallest stem diameter (3.2 mm) was measured in *C. raveyi* under semi controlled greenhouse conditions. Karagüzel et al. (2006) suggested that the stem diameter of *C. orientalis* grown in native ecological conditions was negatively affected by high temperature, pH (≤ 8.3), potassium level and calcium content. However, altitude, organic matter, soil texture, EC value phosphorus and magnesium content contributed to the increase of the stem diameter. The average stem diameter was measured and it was 4.9 mm in Cevizli and 4.3 mm in Korkuteli. These results were similar to that of the current study as average stem diameter in *C. orientalis* was around 5.6 mm but for *C. raveyi*, the stem diameter was considerably low. Karagüzel et al. (2007) explained that growing conditions had significant impacts on stem diameter. On the other hand, there were no significant differences between sowing time and interaction among sowing time and growing condition for stem diameter. Stem diameter of *C. orientalis* which were grown under unheated greenhouse conditions in December showed highest value (9.2 mm) and it showed lowest value (6.0 mm) in February. Stem diameter for plants which was grown in the open field in October, Nowember and December was measured between 1.2 mm-1.9 mm. These results agree with the current study.

Table 4.20. The average values of stem diameter of species growing under semi controlled greenhouse condition

Source		Mean
Species		
C. orientalis		5.6a
C. raveyi		3.2b
Year		
2018		4.4
2019		4.4
Interaction		
C. orientalis	2018	5.7
C. raveyi	2018	3.1
C. orientalis	2019	5.5
C. orientalis	2019	3.3
Overall		4.4

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.3 The length of the main inflorescence

The length of the main inflorescence was measured at the fully flowered stage. The ANOVA for the main inflorescence length for the species under study is given in Table 4.21. The results indicated that there were significant differences between species in terms of their main inflorescence length.

Table 4.21. ANOVA table for main inflorescence length of different species growing under semi controlled greenhouse condition

		Main inflorescence
		Length (cm)
Source	**Degree of freedom**	**Mean Square**
Species (S)	1	1096*
Replication / R	4	6
Year (Y)	1	6
S x Y	1	47
Error	4	8

*indicates significance at $p \leq 0.05$.

The average value of the main inflorescence length is given in Table 4.22. *C. orientalis* showed the highest main inflorescence length (63.1 cm) as compared to *C. raveyi* (44.0 cm). Karagüzel et al. (2006) reported that main inflorescence length was 46.5 cm in Korkuteli and 52.4 cm in Cevizli condition. Another study conducted to determine the effect of growing conditions and sowing time on *C. orientalis* population by Karagüzel et al. (2007) showed significant differences related with the main inflorescence length among growing conditions and sowing time. The highest main inflorescence length (65.9 cm) was observed at unheated greenhouse condition. Furthermore, the results also showed that the delay in sowing date caused decrease in the length of the main inflorescence under open field and unheated greenhose conditions. Karagüzel and Mansuroğlu (2003) investigated the effects of floral spray of GA_3 on *C. orientalis* and found that, the main inflorescence length significantly increased after treatment with 250 ppm GA_3 (94.3 cm) and 500 ppm (98.4 cm) GA_3 as compared to the control group (73.9 cm). The results of the current study shows that the main inflorescence length results are in accordance with previous studies.

Table 4.22. The average values of main inflorescence length of species growing under semi controlled greenhouse condition

		Main inflorescence
		Length (cm)
Source		**Mean**
Species		
C. orientalis		63.1a
C. raveyi		44.0b
Year		
2018		54.2
2019		52.8
Interaction		
C. orientalis	2018	61.8
C. raveyi	2018	46.6
C. orientalis	2019	64.4
C. raveyi	2019	41.3
Overall		53.5

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.4 The flower number of main inflorescences

The number of flowers in the main inflorescence was recorded at fully flowered stage. The ANOVA for the flower number of the main inflorescence is given in Table 4.23. Significant differences were observed for interaction between species and years.

Table 4.23. ANOVA table for the flower number of main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence
		Flower number
Source	**Degree of freedom**	**Mean Square**
Species (S)	1	6.2
Replication / R	4	4.7
Year (Y)	1	0.3
S x Y	1	112.9*
Error	4	4.4

*indicates significance at $p \leq 0.05$.

The average value of number of flowers in the main inflorescence is given in Table 4.24. *C. orientalis* and *C. raveyi* which were grown in 2018 and 2019 showed significant differences regarding the number of flowers in the main inflorescence. Number of flowers were averaging between 32.3-38.7 for *C. orientalis* and 37.0-31.2 for *C. raveyi* between 2018 and 2019 respectively. The number of flowers in the main inflorescence were 23.2 in Korkuteli and 26.6 in Cevizli as explained by Karagüzel et al. (2006). Karagüzel et al (2007) suggested that there were significant differences between open field and unheated greenhouse conditions regarding the number of flowers in the main inflorescence. The number of flowers increased (31.4-36.1) under unheated greenhouse conditions while there ws a decrease (7.0-11.6) in response to open field condition depending on the different sowing dates. Karagüzel and Mansuroğlu (2003) investigated the effect of floral spray of GA_3 on *C. orientalis* and found the highest number of flowers in the main infloresence (20.2) to be at 500 ppm GA_3 (33.1) as compared to 250 ppm GA_3 (29.6) and the control group (25.1). The outcomes of our study demostrated similar results.

Table 4.24 The average values for number of flower of main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence
		Flower number
Source		**Mean**
Species		
C. orientalis		35.5
C. raveyi		34.1
Year		
2018		34.7
2019		35.0
Interaction		
C. orientalis	2018	32.3b
C. raveyi	2018	37.0a
C. orientalis	2019	38.7a
C. raveyi	2019	31.2b
Overall		34.8

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.5 The flower length and diameter in the main inflorescence

The flower length and diameter in the main inflorescence was recorded at fully flowered stage. The ANOVA regarding the flower length and diameter in the main inflorescence of the different species are given in Table 4.25. The results indicated that the flower length and diameter in the main inflorescence were found to be significant in terms of species, over the study years and interaction between species and years.

Table 4.25. ANOVA table for the flower length and diameter in main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence	
		Flower length (mm)	Flower diameter (mm)
Source	Degree of freedom	Mean square	Mean square
Species (S)	1	109.3*	562.1*
Replication / R	4	1.1	1.3
Year (Y)	1	26.2*	19.6*
S x Y	1	11.1*	15.0*
Error	4	0.5	0.4

*indicates significance at $p \leq 0.05$.

The average values of flower length and diameter in the main inflorescence are given in Table 4.26. The significant differences for flower length and diameter were observed for the species, years and between species and years. The specie *C. orientalis* showed the highest flower length (24.7 mm) and diameter (31.9 mm) while *C. raveyi* had the lowest flower length (18.7 mm) and diameter (18.2 mm). Flower length was found to be significant with an average of 23.2 mm in 2018 and 20.2 mm in 2019. In addition, flower diameter was found to be significant with an average of 23.8 mm in 2019. Furthermore, the interaction between species and years of sowing (2018-2019) were founded to be significant. In the year 2018, the species used in the study showed higher values for morphological traits as compare to those for 2019. Karagüzel ve Mansuroğlu (2003) indicated that flower diameter in the main inflorescence in *C. orientalis* was found to be significant at 500 ppm GA_3 (2.9 cm) as compared to 250 ppm GA_3 (2.4 cm) and the control group (2.1 cm). In addition, flower length was found to be significant at 500 ppm GA_3 (2.6 cm) as compared to 250 ppm GA_3 (2.4 cm) and the control group (2.1 cm). This study results were found to be similar with that of previous studies.

Table 4.26. The average values of the flower length and diameter in main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence	
		Flower length (mm)	**Flower diameter (mm)**
Source		**Mean**	**Mean**
Species			
C. orientalis		24.7a	31.9a
C. raveyi		18.7b	18.2b
Year			
2018		23.2a	26.3a
2019		20.2b	23.8b
Interaction			
C. orientalis	2018	27.2a	34.3a
C. raveyi	2018	19.2a	18.4a
C. orientalis	2019	22.3b	29.5b
C. raveyi	2019	18.2b	18.1b
Overall		21.7	25.1

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.6 The pedicel length in main inflorescence

The pedicel length was measured from the lower, middle and upper part of the main inflorescence at a fully flowered stage. The ANOVA for pedicel length in the main inflorescence is given in Table 4.27. The results showed that there were no significant differences for the consecutive years and the interaction between years and specie regarding the pedicel length.

Table 4.27. ANOVA table for the pedicel length in main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence		
		Lower pedicel length(mm)	**Middle pedicel length (mm)**	**Upper pedicel length (mm)**
Source	**Degree of freedom**	**Mean Square**	**Mean Square**	**Mean Square**
Species (S)	1	---	---	---
Replication / P	4	104.7	3.2	0.04
Year (Y)	1	9.2	2.5	1.6
S x Y	1	---	---	---
Error	4	30.7	0.59	0.14

*indicates significance at $p \leq 0.05$.

The average value of pedicel length in the main inflorescence is given in Table 4.28. The pedicel length which was measured from the lower, middle and upper part of the main inflorescence in *C. orientalis* were found to be 74.6 mm, 19.1 mm and 8.4 mm, respectively. There were no pedicel observed in *C. raveyi*. In addition, there were no significant differences among years and interaction between species and years. Karagüzel ve Mansuroğlu (2003) reported that the pedicel length was significantly affected by treatment with 250 ppm GA_3 (4.7 cm) and 500 ppm (5.7 cm) GA_3 as compared to the control group (1.7 cm). The current study indicated that the pedicel length was higher in comparison with the study conducted by Karagüzel ve Mansuroğlu (2003).

Table 4.28. The average values of the pedicel length of main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence		
		Lower pedicel length (mm)	**Middle pedicel length (mm)**	**Upper pedicel length (mm)**
Source		**Mean**	**Mean**	**Mean**
Species				
C. orientalis		74.6	19.1	8.4
C. raveyi		---	---	---
Year				
2018		73.3	19.8	8.4
2019		75.8	19.5	7.5
Interaction				
C. orientalis	2018	73.3	19.8	8.4
C. raveyi	2018	---	---	---
C. orientalis	2019	75.8	18.5	7.3
C. raveyi	2019	---	---	---
Overall		74.6	19.3	8.0

4.2.2.7 The internode length in main inflorescence

The internode length was measured from the lower, middle and upper part of the main inflorescence during full flowered stage. The ANOVA for internode length in the main inflorescence is given in Table 4.29. The results indicated that there were significant differences between species for the lower, middle and upper internodes length in the main inflorescence.

Table 4. 29. ANOVA table for internode length in main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence		
		Lower internode length(mm)	**Middle internode length(mm)**	**Upper internode length(mm)**
Source	**Degree of freedom**	**Mean Square**	**Mean Square**	**Mean Square**
Species (S)	1	2736.7*	145.3*	68.9*
Replication / P	4	0.7	4.6	0.75
Year (Y)	1	1.9	0.1	1.1
S x Y	1	0.3	3.8	0.07
Error	4	0.9	0.7	0.25

*indicates significance at $p \leq 0.05$.

The average value of internode length in the main inflorescence of different species grown under semi controlled greenhouse conditions are given in Table 4.30. The internodes length which were measured from the lower, middle and upper part of the main inflorescence of *C. orientalis* significantly had the highest value compared to *C. raveyi*. Karagüzel and Mansuroğlu (2003) reported that internode lengths were increased with application of 500 ppm GA_3 (3.7 cm) and 250 ppm GA_3 (3.3 cm) GA_3 as compared to the control group (2.4 cm). These results were similar to the current study as the average of the lower, middle and upper internodes lengths in *C. orientalis* were around 50.3 mm 22.3 mm and 10.6 mm respectively. However, the average value of the lower, middle and upper internode length in *C. raveyi* was considerably low.

Table 4.30. The average values of internode length in main inflorescence of different species growing under semi controlled greenhouse condition

		Main inflorescence		
		Lower internode length (mm)	**Middle internode length (mm)**	**Upper internode length (mm)**
Source		**Mean**	**Mean**	**Mean**
Species				
C. orientalis		50.3a	22.3a	10.6a
C. raveyi		19.5b	15.3b	6.3b
Year				
2018		35.0	18.7	9.0
2019		34.2	18.5	8.4
Interaction				
C. orientalis	2018	49.9	22.7	11.4
C. raveyi	2018	20.1	14.6	6.5
C. orientalis	2019	49.5	21.8	10.7
C. raveyi	2019	18.9	15.9	6.0
Overall		34.8	18.8	8.6

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.8 Number of secondary branch

Number of secondary branches were measured at full flowered stage. The ANOVA for number of secondary branches are given in Table 4.31. The results indicated that there were significant differences between species regarding number of secondary branches.

Table 4.31. ANOVA table for number of secondary branches of different species growing under semi controlled greenhouse condition

		Secondary branches
Source	**Degree of freedom**	**Mean Square**
Species (S)	1	15.0*
Replication / P	4	0.3
Year (Y)	1	0.1
S x Y	1	0.5
Error	4	0.2

*indicates significance at $p \leq 0.05$.

The average values of number of secondary branches are given in Table 4.32. The highest number of secondary branches were observed with an average of 5.8 per plant in *C. orientalis* compared to *C. raveyi*. *C. raveyi* had the lowest number of secondary branches with an average of 3.6 per plant. Karagüzel et al. (2006) reported that, the number of secondary branches were measured at 3.9 cm in Korkuteli and 4.5 cm in Cevizli conditions. It suggests that different ecological conditions caused these significant differences. Karagüzel et al. (2007) pointed out that, the number of secondary branches in *C. orientalis* were found to be significant for open field and unheated greenhouse conditions. The number of secondary branches were recorded (6.8-5.8) with no significant differences in terms of sowing time under unheated greenhouse conditions. On the other hand, the lowest number of secondary branches were observed (1.3-1.5) with no significant differences regarding sowing time in the open field condition. The results related with the number of secondary branches were in line with the current study. Karagüzel ve Mansuroğlu (2003) indicated that there were no significant changes observed among treatment with 250 ppm (9.5) and 500 ppm (9.5) GA_3 as compared to the control group (9.1) regarding the number of secondary branches in *C. orientalis*. However, the number of secondary branches in *C. raveyi* was considerably low compared to the result of Karagüzel ve Mansuroğlu (2003).

Table 4.32. The average values of number of secondary branches of different species growing under semi controlled greenhouse condition

		Secondary brances
Source		**Mean**
Species		
C. orientalis		5.8a
C. raveyi		3.6b
Year		
2018		4.7
2019		4.8
Interaction		
C. orientalis	2018	6.0
C. raveyi	2018	3.3
C. orientalis	2019	5.7
C. raveyi	2019	3.9
Overall		4.7

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.9 Length of secondary branch

The length of secondary branches which were positioned in the lower, middle and upper part of the stem were measured at fully flowered stage. The ANOVA regarding secondary branches length is given in Table 4.33. The results indicated that there were significant differences among species and interaction between species and years regarding length of secondary branches. Moreover, the upper length of secondary branches were found to be significant for consecutive years.

Table 4.33. ANOVA table for length of secondary branches of different species growing under semi controlled greenhouse condition

		Secondary branches		
		Lower length(cm)	Middle length (cm)	Upper length (cm)
Source	**Degree of freedom**	**Mean Square**	**Mean Square**	**Mean Square**
Species (S)	1	150.6*	1150*	2707*
Replication / P	4	6.4	1	6
Year (Y)	1	4.1	1	123*
S x Y	1	37.3*	49*	165*
Error	4	2.3	2	13

*indicates significance at $p \leq 0.05$.

The average values related with length of secondary branches are given in Table 4.34. The length of secondary branches which were positioned in the lower, middle and upper part of the stem was found to be significant among species. The length of secondary branches in lower, middle and upper part of stem were measured 24.9 cm, 44.8 cm and 54.3 cm in *C. orientalis*, respectively. Alongside, the length of secondary branches in lower, middle and upper part of stem were measured 17.8 cm, 25.2 cm and 24.2 cm. in *C. raveyi*, respectively. These findings showed that the branches which were positioned at upper part of stem were longer than branches positioned at the lower part of stem in *C. orientalis*. However, the middle and upper length of secondary branches were approximately similar with averages of 25.2 cm and 24.2 cm in *C. raveyi*, respectively. In addition, differences were observed regarding the upper length of secondary branches for consecutive years. In the year 2018, the upper length of secondary branches were higher as compared to 2019. Furthermore, species interaction with years were found to be significant regarding length of secondary branches. The lower, middle and upper length of secondary branches in *C. orientalis* were measured as 23.7 cm, 43.1 cm and 53.8 cm in 2018. On the other hand, the lower, middle and upper length of secondary branches in *C. orientalis* was found to be higher with averages of 26.1 cm, 46.5 cm and 54.8 cm in 2019, respectively. However, these traits which were measured in 2019 were considerably lower than 2018 for *C. raveyi*. The lower, middle and upper length of secondary branches were recorded with averages of 20.2 cm, 27.5 cm and 31.1 cm for *C. raveyi* in 2018, respectively. Moreover, the lower, middle and upper length of

secondary branches were recorded with averages of 15.5 cm, 22.9 cm and 17.3 cm for *C. raveyi* in 2019, respectively. Karagüzel et al. (2006) explained that length of secondary branches in *C. orientalis* was measured with averages of 31.1 cm in Korkuteli, while these traits were observed at 35.5 cm in Cevizli. Karagüzel et al. (2007) reported that secondary branches length which was measured in unheated greenhouse (50,2 cm-54,3 cm) during all sowing time was higher than the open field condition (7,4 cm-12,3 cm). Karagüzel and Mansuroğlu (2003) indicated that secondary branches length was increased after treatment with 250 ppm GA_3 (39.6 cm) and 500 ppm GA_3 (42.3 cm) compared to the control group (32.1 cm). The results of the current study demostrated that length of secondary branches are in accordance with previous studies.

Table 4.34. The average values of length of secondary branches of species growing under semi controlled greenhouse condition

		Secondary branches		
Source		**Lower length (cm) Mean**	**Middle length (cm) Mean**	**Upper length (cm) Mean**
Species				
C. orientalis		24.9a	44.8a	54.3a
C. raveyi		17.8b	25.2b	24.2b
Year				
2018		22.0	35.3	42.4a
2019		20.8	34.7	36.0b
Interaction				
C. orientalis	2018	23.7a	43.1a	53.8a
C. raveyi	2018	20.2a	27.5a	31.1a
C. orientalis	2019	26.1b	46.5b	54.8b
C. raveyi	2019	15.5b	22.9b	17.3b
Overall		21.4	35.0	39.2

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.10 Length of secondary inflorescence

The length of secondary inflorescences which were positioned at the lower, middle and upper part of the stem were measured at fully flowered stage. The ANOVA regarding length of secondary inflorescences of different species are given in Table 4.35. The results indicated that there were significant differences among species regarding the length of secondary inflorescences positioned in the middle and upper part of the stem. Also, the length of secondary inflorescences which were positioned at the lower and middle part of the stem had significant differences for the study years. Furthermore, species and years interaction were found to be significant.

Table 4.35. ANOVA table for length of secondary inflorescences of different species growing under semi controlled greenhouse condition

Source		Secondary inflorescence		
		Lower lenght (cm)	Middle lenght (cm)	Upper lenght (cm)
Significance	Degree of freedom	Mean Square	Mean Square	Mean Square
Species (S)	1	3.5	107*	747*
Replication / R	4	7.4	4	7
Year (Y)	1	1.5*	37*	19
S x Y	1	19.7*	66*	120*
Error	4	1.1	4	7

*indicates significance at $p \leq 0.05$.

The average length of secondary inflorescences of different species grown under semi controlled greenhouse conditions are given in Table 4.36. The differences between species were observed among length of secondary inflorescences which were positioned in the middle and upper part of the stem. The length of secondary inflorescence in the middle part of the stem was measured with an average of 23.8 cm in *C. orientalis* whereas, these trait were observed as 17.8 cm in *C. raveyi*. On the other hand, the length of secondary inflorescence in the upper part of the stem were found to be higher (30.5 cm) for *C. orientalis* as compared to *C. raveyi* (14.7 cm). When the year data was examined, length of secondary branches in the lower and middle part of the stem were averaged between 12.5 cm and 22.5 cm in 2018, respectively. However, these traits

were found to be low with averages of 11.8 cm, and 19.0 cm in 2019, respectively. Furthermore, species interaction with years were found to be significant for the study years. The length of secondary inflorescence in the lower, middle and upper part of the stem were mesaured as 11.7 cm, 23.2 cm and 28.6 cm in 2018 for *C. orientalis*. Whereas, the length of secondary inflorescences in the lower, middle and upper part of the stem were found to be higher with averages of 13.6 cm, 24.4 cm and 32.4 cm in 2019 for *C. orientalis*, respectively. In addition, the length of secondary inflorescences in the lower, middle and upper part of the stem were recorded with averages of 13.2 cm, 21.9 cm and 19.1 cm for *C. raveyi* in 2018, respectively. Nevertheless, the length of secondary branches in the lower, middle and upper part of the stem were measured with averages of 10.2 cm, 13.7 cm and 10.3 cm for *C. raveyi* in 2019, respectively. These findings which were recorded in 2019 were remarkably low compared to 2018 in terms of *C. raveyi*. The secondary inflorescence of species under study were shorter than the main inflorescence in the current study. The length of the main inflorescence was measured with an average of 63.1 cm in *C. orientalis* and 44.0 cm in *C. raveyi*. The values regarding length of secondary inflorescences were quite lower than length of the main inflorescence which were investigated by Karagüzel and Mansuroğlu (2003), Karagüzel et al (2006) and Karagüzel et al. (2006).

Table 4.36. The average values of length of secondary inflorescences of species growing under semi controlled greenhouse condition

		Secondary inflorescence		
		Lower length (cm)	**Middle length (cm)**	**Upper length (cm)**
Source		**Mean**	**Mean**	**Mean**
Species				
C. orientalis		12.7	23.8a	30.5a
C. raveyi		11.6	17.8b	14.7b
Year				
2018		12.5a	22.5a	23.8
2019		11.8b	19.0b	21.3
Interaction				
C. orientalis	2018	11.7a	23.2a	28.6a
C. raveyi	2018	13.2a	21.9a	19.1a
C. orientalis	2019	13.6b	24.4b	32.4b
C. raveyi	2019	10.0b	13.7b	10.3b
Overall		12.1	20.8	22.6

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.11 Number of flowers in secondary inflorescence

The number of flowers in secondary inflorescence which were positioned in the lower, middle and upper part of the stem were measured at full flowering stage. The ANOVA regarding the number of flowers in secondary inflorescences of different species are given in Table 4.37. The results indicated that there were significant differences between species for the number of flowers in secondary inflorescences which were positioned in the middle and upper part of the stem. In addition, the species and year interaction were found to be significantly related with number of flowers in the secondary inflorescences which were positioned at the middle and upper part of the stem.

Table 4.37. ANOVA table for number of flowers in secondary inflorescences of different species growing under semi controlled greenhouse condition

Source		Secondary inflorescence		
		Lower flower number	Middle flower number	Upper flower number
Significance	Degree of freedom	Mean Square	Mean Square	Mean Square
Species (S)	1	0.2	52.1*	253.9*
Replication / R	4	1.3	5.7	5.6
Year (Y)	1	4.2	0.3	1.8
S x Y	1	5.2	3.4	32.7*
Error	4	0.9	4.6	1.4

*indicates significance at $p \leq 0.05$.

The average value of the number of flowers at secondary inflorescences is given in Table 4.38. The differences between species were observed for the number of flowers in secondary inflorescences which were positioned at the middle and upper part of the stem. The number of flowers of secondary inflorescences in the middle and upper part of the stem were recorded with averages of 15.5 and 19.9 for in *C. orientalis*. However, the number of flowers which were obtained for these traits were lower in *C. raveyi*. The number of flowers of secondary inflorescences in the middle and upper part of the stem were averaged between 11.4 and 10.7 for *C. raveyi*, respectively. Furthermore, species interaction with year regarding number of flowers in the secondary inflorescence which were positioned at the upper part of the stem were found to be significant for the consecutive year. This trait which was measured in 2019 was higher as compared to 2018 for *C. orientalis*. However, it was lower in the year 2019 as compared to 2018 for *C. raveyi*. Karagüzel et al (2007) pointed out that the highest results related with the number of flowers in secondary inflorescence of *C. orientalis* were obtained under untheated greenhouse conditions in October (28.4), November (31.5) and December (31.9). Though, the number of flowers in the secondary inflorescences of *C. orientalis* grown under the open field condition were found considerably low in October (5.1), November (5.8) and December (2.9). They also explained that these differences in the number of flowers in secondary branches were as a result of growing conditions. Karagüzel et al. (2006) reported that, the number of flowers in secondary branches were measured with an average of 13.8 in Korkuteli and 15.9 in Cevizli. These investigations

done by Karagüzel et al (2006) indicated that ecological conditions affected the number of flowers in secondary inflorescences of *C. orientalis*. Karagüzel and Mansuroğlu (2003) Karagüzel and Mansuroğlu (2003) indicated that, the number of flowers in secondary inflorescences of *C. orientalis* increased after treatment with 250 ppm GA_3 (20.2) and 500 ppm GA_3 (17.2) as compared to the control group (14.9). The results of this current study are similar to that of previous studies.

Table 4.38. The average values of number of flowers in secondary inflorescences of different species of species growing under semi controlled greenhouse condition

		Secondary inflorescence		
		Lower flower number	**Middle flower number**	**Upper flower number**
Source		**Mean**	**Mean**	**Mean**
Species				
C. orientalis		8.9	15.5a	19.9a
C. raveyi		8.6	11.4b	10.7b
Year				
2018		9.3	13.3	15.7
2019		8.2	13.6	15.0
Interaction				
C. orientalis	2018	8.8	14.8	18.7b
C. raveyi	2018	9.9	11.7	12.8a
C. orientalis	2019	9.0	16.2	21.2a
C. raveyi	2019	7.4	11.0	8.7b
Overall		8.8	13.5	15.3

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.12 The flower length and diameter in secondary inflorescence

The flower length and diameter in secondary inflorescences were measured at fully flowered stage. The ANOVA regarding number of flowers in secondary inflorescences of the different species are given in Table 4.39. The results indicated that there were significant differences between species.

Table 4.39. ANOVA table for flower length and diameter in secondary inflorescences of different species growing under semi controlled greenhouse condition

Source		Secondary inflorescence	
		Flower length (mm)	Flower diameter (mm)
Significance	Degree of freedom	Mean Square	Mean Square
Species (S)	1	297*	154.1*
Replication / R	4	0.2	0.5
Year (Y)	1	1.7	0.3
S x Y	1	0.02	0.02
Error	4	0.09	0.3

*indicates significance at $p \leq 0.05$.

The average value of flower length and diameter in secondary inflorescences of different species grown under semi controlled greenhouse condition are given in Table 4.40. The flower length and diameter in secondary inflorescences were measured with averages of 27.9 mm and 24.3 mm in *C. orientalis*. However, these traits were found to be lower for *C. raveyi*. The flower length and diameter of secondary inflorescences were observed with averages of 17.8 mm and 18.0 mm in *C. raveyi*. These results indicated that the flowers of secondary inflorescences were seen smaller than the flowers of the main inflorescence for both species. Karagüzel and Mansuroğlu (2003) reported that the flower diameter in the main inflorescences in *C. orientalis* were found to be significant after treatment with 500 ppm GA_3 (2.9 cm) compared to 250 ppm GA_3 (2.4 cm) and the control group (2.1 cm). The results of this current study showed that it agrees with previous studies.

Table 4. 40. The average values of flower length and diameter in secondary inflorescences of different species of species growing under semi controlled greenhouse condition

		Secondary inflorescence	
		Flower length (mm)	**Flower diameter (mm)**
Source		**Mean**	**Mean**
Species			
C. orientalis		27.9a	24.3a
C. raveyi		17.8b	18.0b
Year			
2018		23.5	21.6
2019		23.1	21.1
Interaction			
C. orientalis	2018	28.1	25.2
C. raveyi	2018	18.1	17.9
C. orientalis	2019	27.3	24.8
C. raveyi	2019	17.4	17.7
Overall		22.9	21.3

Different letters indicate significant differences in by Tukey test at 0.05.

4.2.2.13 The pedicel length in secondary inflorescence

The pedicel length in secondary inflorescences were measured from the lower, middle and upper part of the stem at full flowering stage. The ANOVA regarding pedicel length in secondary inflorescences of the different species are given in Table 4.41. The results showed that there were no significant differences for the consecutive years and there were no interactions between years and species regarding the pedicel length in main secondary inflorescences.

Table 4.41. ANOVA table for pedicel length in secondary inflorescences of different species growing under semi controlled greenhouse condition

		Secondary inflorescence		
Source		**Lower pedicel length (mm)**	**Middle pedicel length (mm)**	**Upper pedicel length (mm)**
Significance	**Degree of freedom**	**Mean Square**	**Mean Square**	**Mean Square**
Species (S)	1	---	---	---
Replication / P	4	1.8	2.1	0.9
Year (Y)	1	2.0	1.0	1.8
S x Y	1	---	---	---
Error	4	0.6	4.2	1.0

*indicates significance at $p \leq 0.05$.

The average values of pedicel length in secondary inflorescence of species grown under semi controlled greenhouse conditions are given in Table 4.42. The pedicel length in secondary inflorescence which were measured from the lower, middle and upper part of stem of *C. orientalis* were found to be 11.6 mm, 13.4 mm and 15.2 mm, respectively. There was no pedicel in *C. raveyi*. In addition, there were no significant differences among years and interaction between species and years. Karagüzel ve Mansuroğlu (2003) reported that the pedicel length significantly affected treatment with 250 ppm GA_3 (4.7 cm) and 500 ppm GA_3 (5.7 cm) compared to the control group (1.7 cm). This current study indicated that the pedicel length was found to be similar with the control group which was obtained by Karagüzel ve Mansuroğlu (2003).

Table 4.42. The average values of pedicel length in secondary inflorescences of different species of species growing under semi controlled greenhouse condition

		Secondary inflorescence		
		Lower pedicel length (mm)	**Middle pedicel length (mm)**	**Upper pedicel length (mm)**
Source		**Mean**	**Mean**	**Mean**
Species				
C. orientalis		11.6	13.4	14.8
C. raveyi		---	---	---
Year				
2018		11.4	13.0	14.3
2019		12.6	13.8	15.4
Interaction				
C. orientalis	2018	11.4	13.0	14.3
C. raveyi	2018	---	---	---
C. orientalis	2019	12.6	13.8	15.4
C. raveyi	2019	---	---	---
Overall		11.6	13.4	14.8

4.2.2.14 The internode length in secondary inflorescence

The internode length of secondary inflorescences which were positioned at the lower, middle and upper part of the stem were measured at full flower stage. The ANOVA regarding secondary inflorescence pedicel length of different species are given in Table 4.43. The results demostrated that there were significant differences for species, consecutive years and interaction between years and species.

Table 4.43. ANOVA table for internode length in secondary inflorescences of different species growing under semi controlled greenhouse condition

		Secondary inflorescence		
		Lower internode length (mm)	**Middle internode length (mm)**	**Upper internode length (mm)**
Source	**Degree of freedom**	**Mean Square**	**Mean Square**	**Mean Square**
Species (S)	1	29.7*	279.5*	245.5*
Replication / R	4	0.9	3.5	0.4
Year (Y)	1	1.7*	0.1	0.03
S x Y	1	2.0*	0.5	0.09
Error	4	0.2	3.8	0.2

*indicates significance at $p \leq 0.05$.

The average value of internode length in secondary inflorescences of species grown under semi controlled greenhouse conditions are given in Table 4.44. The differences were observed between species regarding internode length in secondary inflorescences which were positioned in the lower, middle and upper part of the stem. The internode length of secondary inflorescences which were positioned at the lower, middle and upper part of the stem were measured with averages of 13.5 mm, 19.9 mm and 19,5 mm for *C. orientalis*, respectively. On the other hand, the internode length of secondary inflorescence in the lower, middle and upper part of the stem was recorded as 9.9 mm, 10.2 mm and 10.5 mm for *C. raveyi*, respectively. Also, the differences were observed for consecutive years regarding internode length of secondary inflorescence in the lower part of the stem for both species. The averages of the internode length of secondary inflorescence in the lower part of the stem was recorded as 11.1 mm in 2018 and 11.8 mm in 2019. Furthermore, interaction among species and years were found to be significant for this trait. The internode length of secondary inflorescence in the lower part of the stem of *C. orientalis* were measured with averages of 12.2 mm in 2018 and 13.8 mm in 2019. Karagüzel and Mansuroğlu (2003) reported that the average internode length in secondary inflorescence of *C. orientalis* was found to be 3.7 mm and 3.3 mm after treatment with 500 ppm GA_3 and 250 ppm GA_3. On the other hand, the control group was measured at 2.4 mm regarding internode length. The results of the current

study showed that the internode length in secondary inflorescences were founded to be lower than those in previous studies.

Table 4.44. The average values of internode in secondary inflorescences of different species of species growing under semi controlled greenhouse condition

		Seconday inflorescence		
		Lower internode length (mm)	**Middle internode length (mm)**	**Upper internode length (mm)**
Source		**Mean**	**Mean**	**Mean**
Species				
C. orientalis		13.5a	19.9a	19.5a
C. raveyi		9.9b	10.2b	10.5b
Year				
2018		11.1b	14.7	14.9
2019		11.8a	15.0	15.0
Interaction				
C. orientalis	2018	12.2b	19.8	19.5
C. raveyi	2018	9.9	10.5	10.6
C. orientalis	2019	13.8a	20.0	19.6
C. raveyi	2019	9.8	9.9	10.4
Overall		11.6	15.0	15.0

Different letters indicate significant differences in by Tukey test at 0.05.

4.3. Investigation of genetic diversity of some species belongs to Ranunculaceae family by using iPBS molecular markers

Plant species belonging to the Ranunculaceae family were investigated to evaluate the relationship with *C. orientalis* and *C. raveyi* which were selected for landscape horticulture studies at NOHU and some parts of the Niğde district.

4.3.1 Genomic DNA extraction

Total genomic DNA was extracted from fresh leaf tissues. The quality and concentration of the extracted DNA was checked using a Shimadzu BioSpec-nano spectrophotometer. Species name, serial number and concentration of extracted DNA is indicated in Table 4.45.

Table 4.45. Concentration of extracted genomic DNA of plant species

Sample ID	Species name	260/280	Concentration (ng/ul)
1	*Ranunculus kotschyi*	1.991	3101.7
2	*Ranunculus repens*	2.150	805.3
3	*Ranunculus cuneatus*	2.137	310.1
4	*Adonis aestivalis* subsp. *aestivalis*	2.008	2158.9
5	*Delphinium venulosum*	2.012	3605.1
6	*Consolida hellespontica*	2.19	1802.3
7	*Consolida regalis* subsp. *paniculata var. paniculata*	2.103	646.7
8	*Consolida thirkeana*	2.001	1404.3
9	*Nigella arvensis* subsp. *glauca*	2.058	1826.8
10	*Consolida orientalis*	2.145	1034.9
11	*Consolida raveyi*	2.109	1200.3

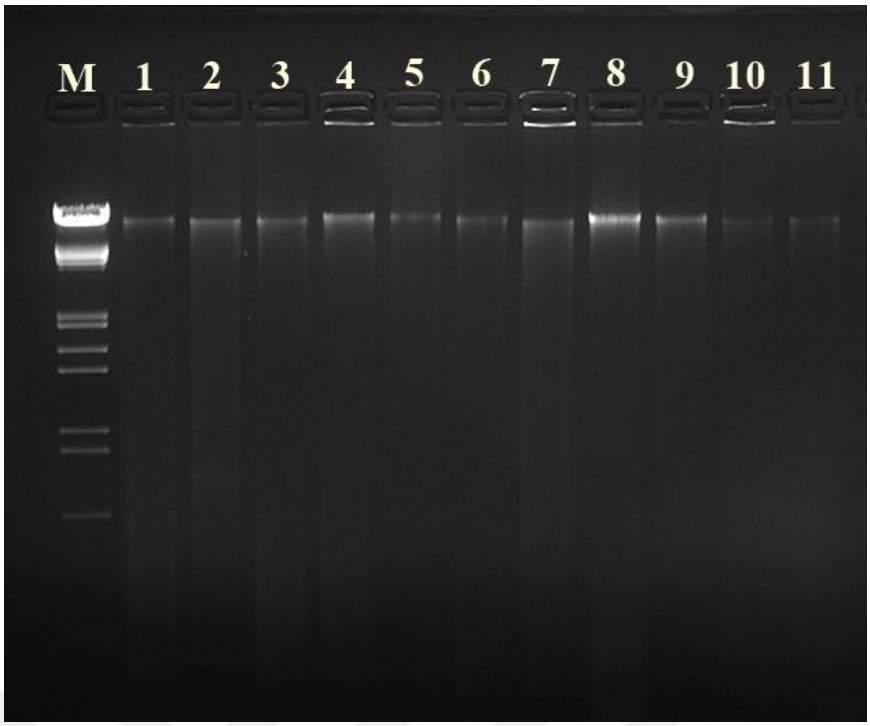

Figure 4.1. Gel picture of extracted DNA of plant species

4.3.2 iPBS screening

This study was conducted to determine the genetic relationship between the 11 plant species that belong to the Ranunculaceae family. 20 Ipbs markers were selected for this purpose. Presence or absence of iPBS bands were recorded (evaluate) '1' or '0', respectively. Scoring of bands were performed manually. All primers which were selected for the determination of genetic similarity, produced scorable bands. Band sizes ranged between 190 to 6000 bp (Figure 4.2, Figure 4.5). 1427 bands were obtained as a result of scoring. 1420 bands were found to be polymorphic among the total bands. For each primer, the total number of scored bands ranged between 50 and 91 and averagely 71.4 bands per primer. Per primer, polymorphic bands ranged between 69 and 90 with an average of 71 bands. The maximum scorable bands obtained using primer 2391, on the other hand, primer 2229 gave less scorable bands. The polymorphism percentage varied from 95.06% to 100% depending on the primer with an average of 99.6% per primer. The PIC value was calculated for each primer that ranged from 0.29 to 0. With the average PIC value of 0.33 for each primer. Primer 2402 gave the highest PIC value of 0.39 (Table 4.46).

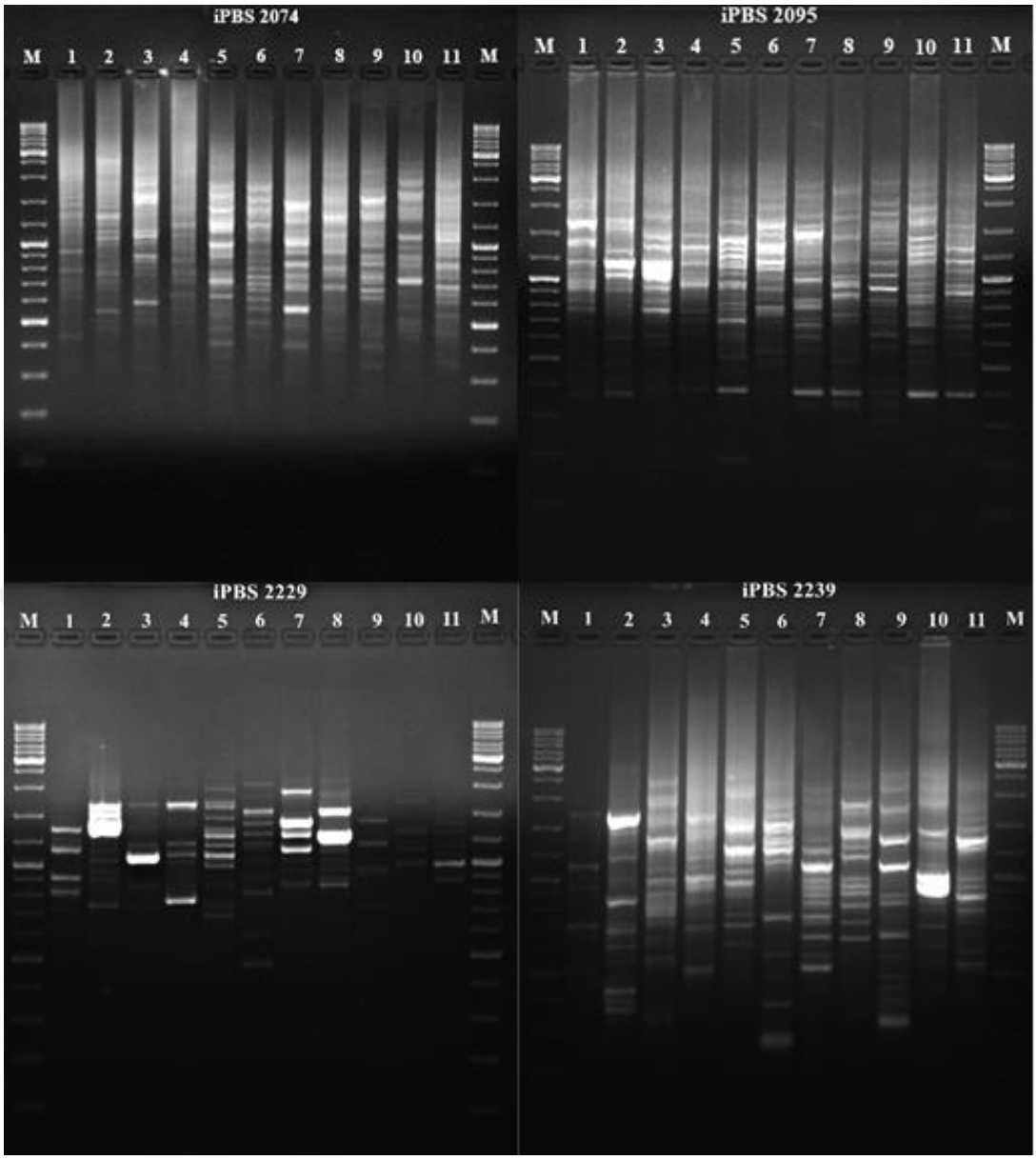

Figure 4.2. Gel results for iPBS Primers, 2074, 2095, 2229, 2239

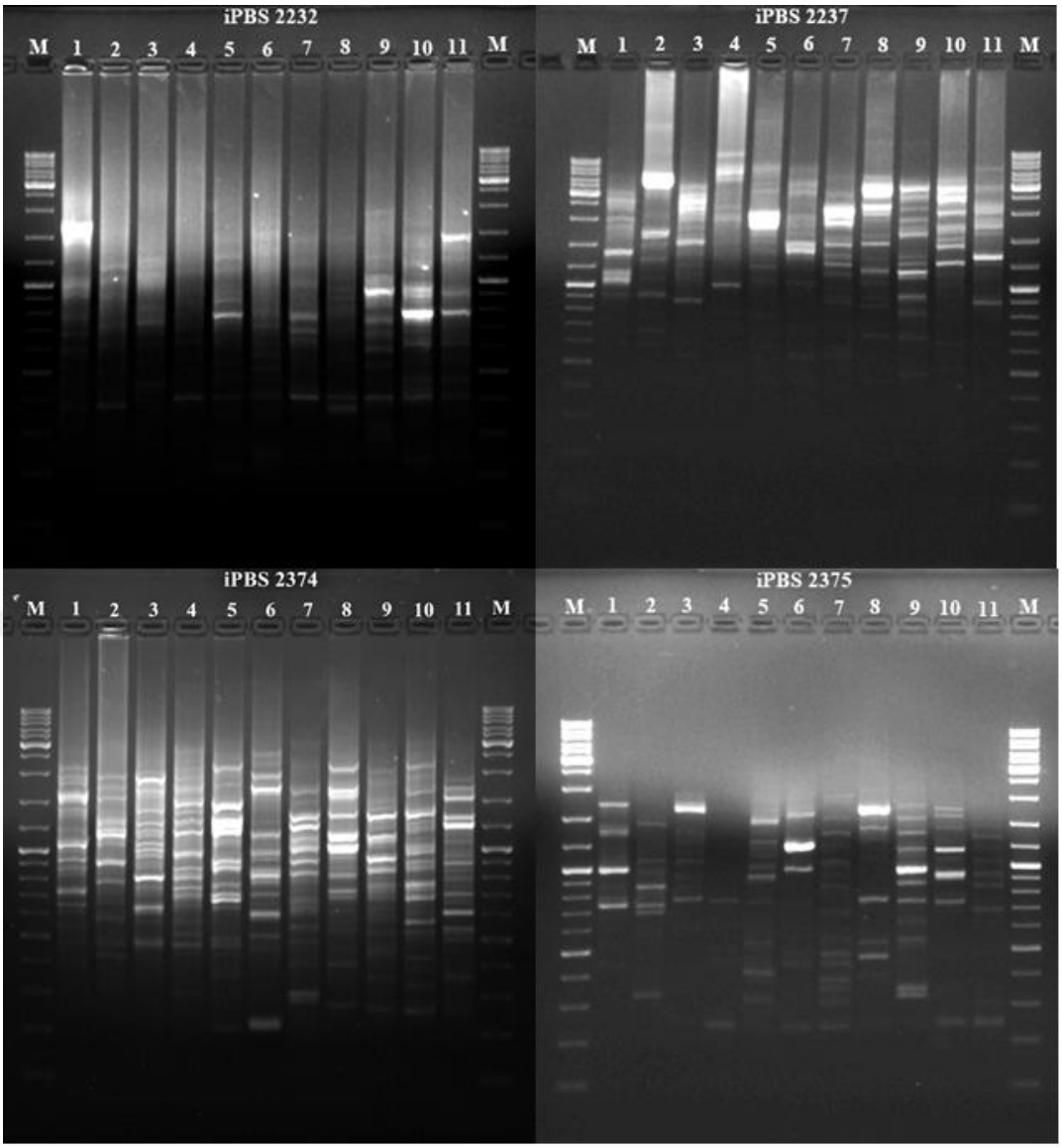

Figure 4.3. Gel results for iPBS Primers, 2232, 2237, 2374, 2375

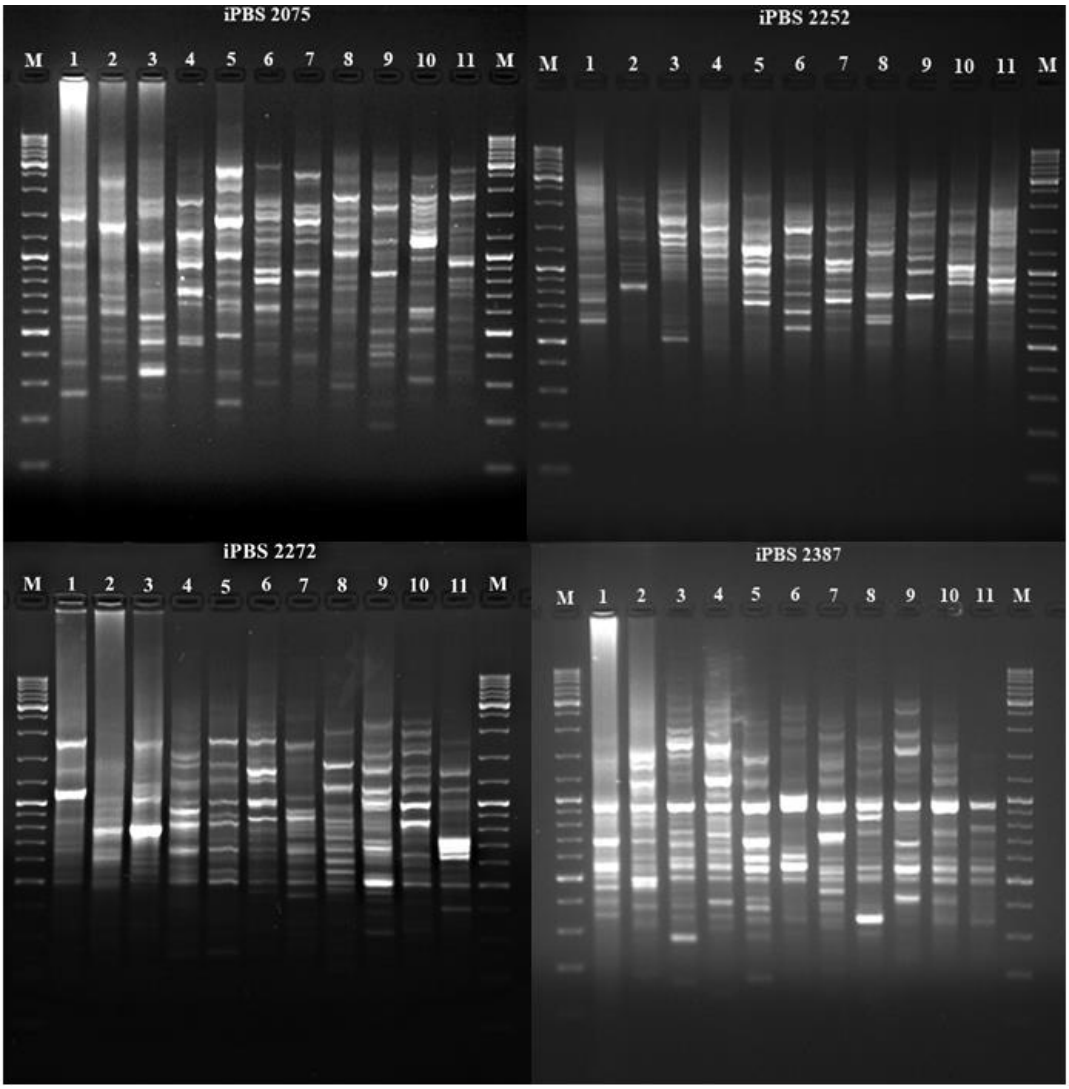

Figure 4.4. Gel results for iPBS Primers, 2075, 2252, 2272, 2387

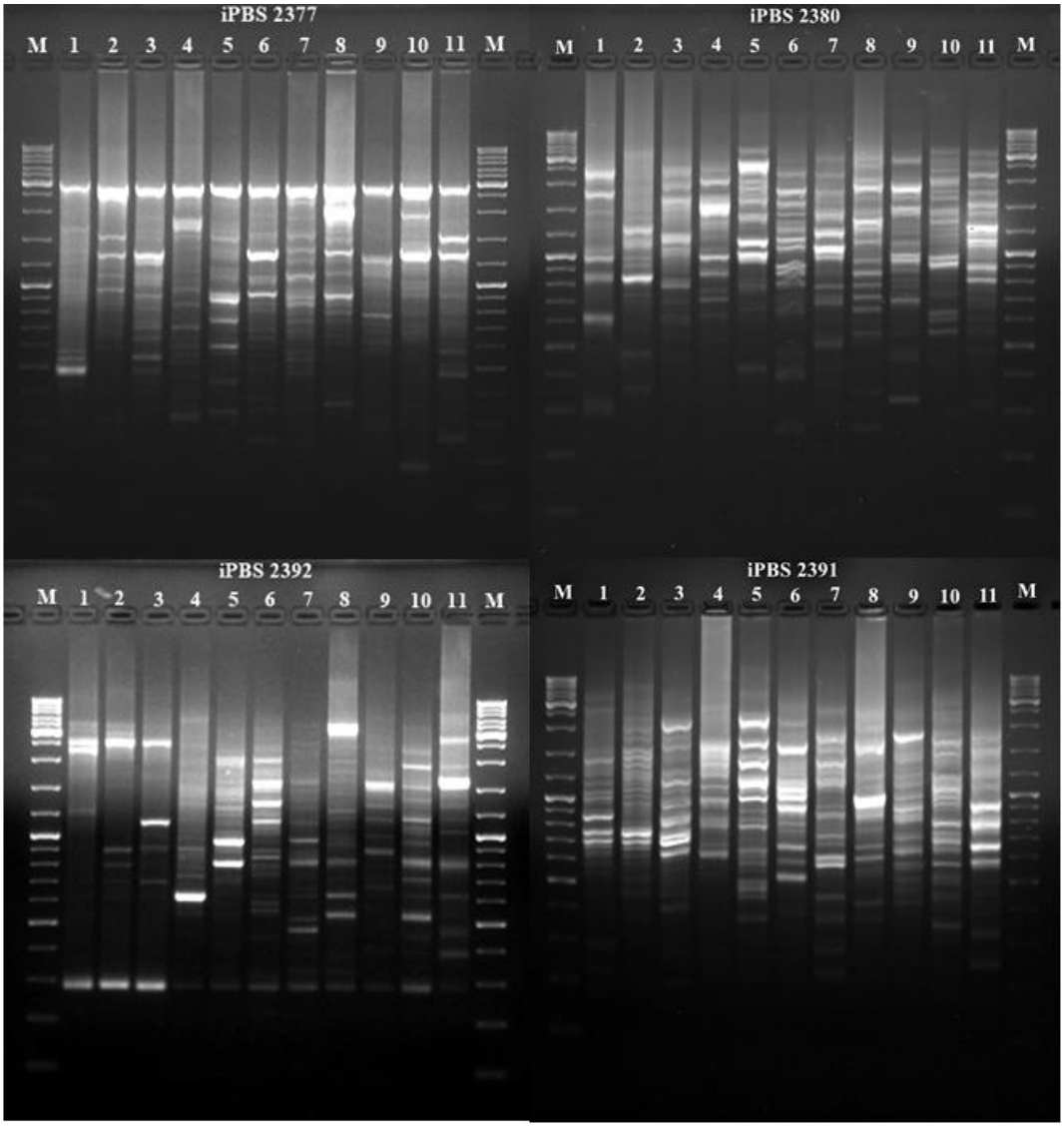

Figure 4.5. Gel results for iPBS Primers, 2377, 2380, 2392, 2391

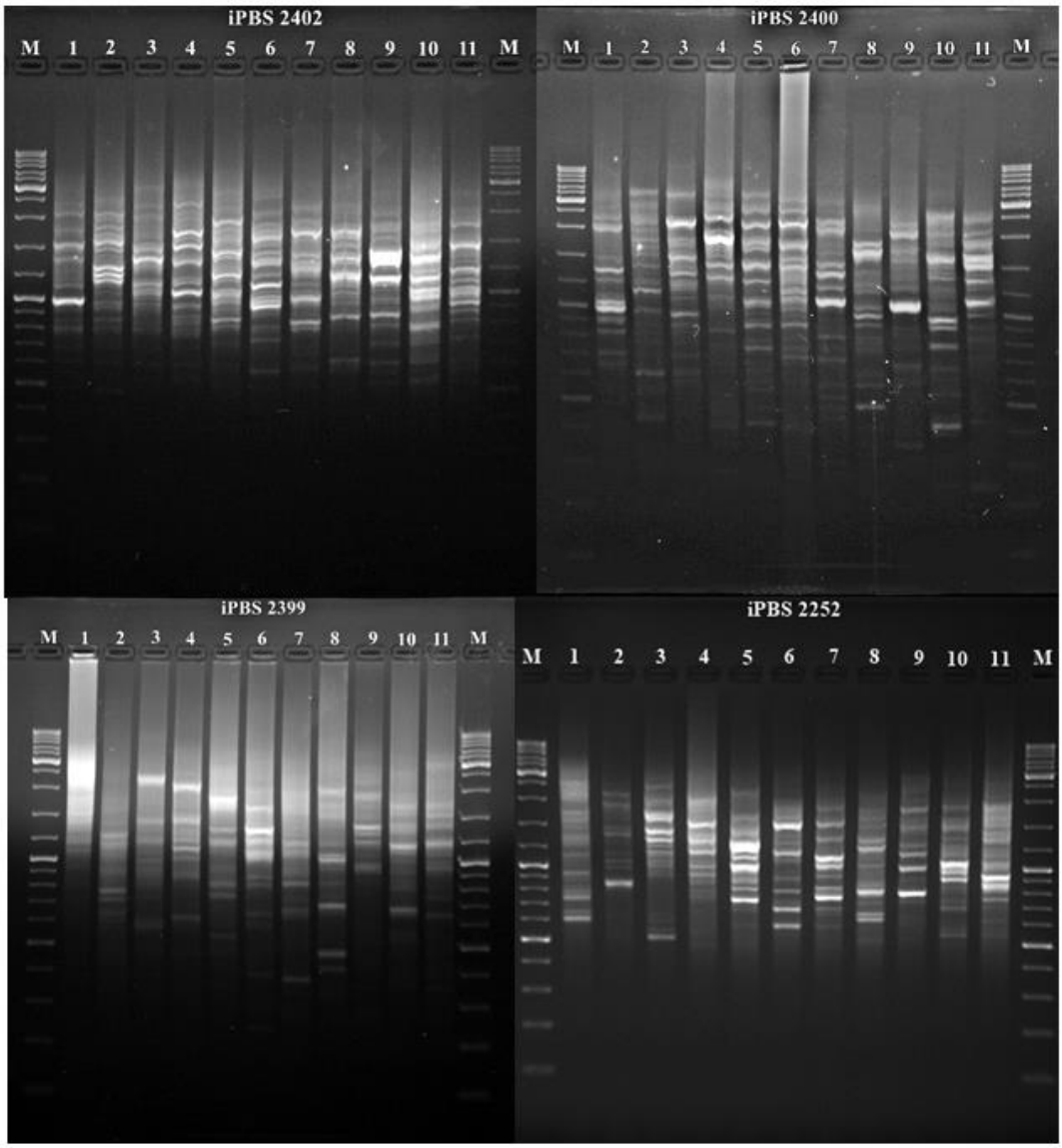

Figure 4.6. Gel results for iPBS Primers, 2402, 2400, 2399, 2252

Table 4.46. Summary of amplified products using ipbs marker system of plants from the Ranunculaceae family

Primer	Scored band sizes (bp)	Number of total scored bands	Number of total polymorphic bands	Percentage of polymorphism (%)	PIC value
2074	350 - 2500	56	56	100%	0.37
2075	190 - 4000	83	83	100%	0.33
2095	270 - 2800	84	84	100%	0.33
2229	480 - 2250	49	49	100%	0.29
2232	220 - 1950	69	69	100%	0.31
2237	500 - 5100	52	52	100%	0.32
2239	290 - 6000	78	78	100%	0.31
2252	470 - 2750	52	52	100%	0.37
2272	230 - 2500	76	75	98.68%	0.33
2274	190 - 5000	87	87	100%	0.29
2374	210 - 3000	90	90	100%	0.33
2375	240 - 2200	52	52	100%	0.30
2380	250 - 3500	88	88	100%	0.31
2387	140 - 3500	81	77	95.06%	0.36
2391	210 - 4000	74	74	100%	0.40
2392	290 - 6000	70	69	98.57%	0.37
2399	390 - 3000	50	50	100%	0.29
2377	190 - 2800	91	90	98.90%	0.30
2400	340 - 3500	84	84	100%	0.32
2402	500 - 2800	61	61	100%	0.39
Total / Mean	---	1427 71.4	1420 71	- 99.6%	- 0.33

The data obtained using iPBS markers were analised by NTSYS program to determine genetic diversity among species. The similarity indexes were calculated among species. The similarity indexes varied from 0.76-0.65 in 11 species. The highest similarity index value (0.76) was recorded between *R. kotschyi* and *R. repens*. On the other hand, *R. repens* and *D. venulosum* had least similarity index (0.65). (Table 4.47).

Table 4.47. Pairwise similarity index comparison matrix of plant samples from the Ranunculaceae family.

Species	*R. kotschyi*	*R. repens*	*R. cuneatus*	*A. aestivalis* subsp. *aestivalis*	*D. venulosum*	*C. hellespontica*	*C. regalis* subsp. *paniculata* var. *paniculata*	*C. thirkeana*	*N. arvensis* subsp. *glauca*	*C. orientalis*	*C. raveyi*
R. kotschyi	1.00										
R. repens	0.76	1.00									
R. cuneatus	0.74	0.74	1.00								
A. aestivalis subsp. *aestivalis*	0.72	0.71	0.72	1.00							
D. venulosum	0.68	0.65	0.67	0.70	1.00						
C. hellespontica	0.69	0.69	0.67	0.68	0.68	1.00					
C. regalis subsp. *paniculata* var. *paniculata*	0.69	0.68	0.66	0.67	0.67	0.68	1.00				
C. thirkeana	0.70	0.67	0.67	0.66	0.66	0.66	0.67	1.00			
N. arvensis subsp. *glauca*	0.69	0.68	0.67	0.66	0.66	0.66	0.66	0.68	1.00		
C. orientalis	0.71	0.68	0.68	0.66	0.65	0.66	0.66	0.67	0.71	1.00	
C. raveyi	0.71	0.71	0.69	0.69	0.66	0.68	0.66	0.69	0.68	0.73	1.00

The cluster analysis was performed utilizing similarity index data which were generated by NTYSS. A dendrogram was obtained by UPGMA method for cluster analysis to indicate genetic similarity of the 11 plant species. The dendrogram revealed that, the similarity coefficent varied from 0.67 to 0.76 (Figure 4.2). Two main clusters occured in the dendrogram. One of the main clusters had *D. venulusum, C. hellespontica, C. regalis* subsp. *paniculata* var. *Paniculata* and the other main cluster was divided into two subclusters. The first subcluster only represented species *C. thirkeana*. The second subcluster was also divided in two small groups, *R. kotschyi, R. repens, R. cuneatus* and *A. aestivalis* subsp. *aestivalis* were grouped into same cluster while *N. arvensis* subsp. *glauca, C. orientalis* and *C. raveyi* were within another subgroup. The species *R. kotschyi, R. repens* and *R. cuneatus* are genetically related whereas these species were distantly related to *D. venulosum, C. hellespontica* and *C. regalis* subsp. *paniculata* var. *paniculata*. In addition, *C. orientalis, C. raveyi* and *N. arvensis* subsp. *glauca* were closely related to each other. Moreover, *D. venulosum* and *C. hellespontica* genotypes were also closely related according to the dendrogram below (Figure 4.7).

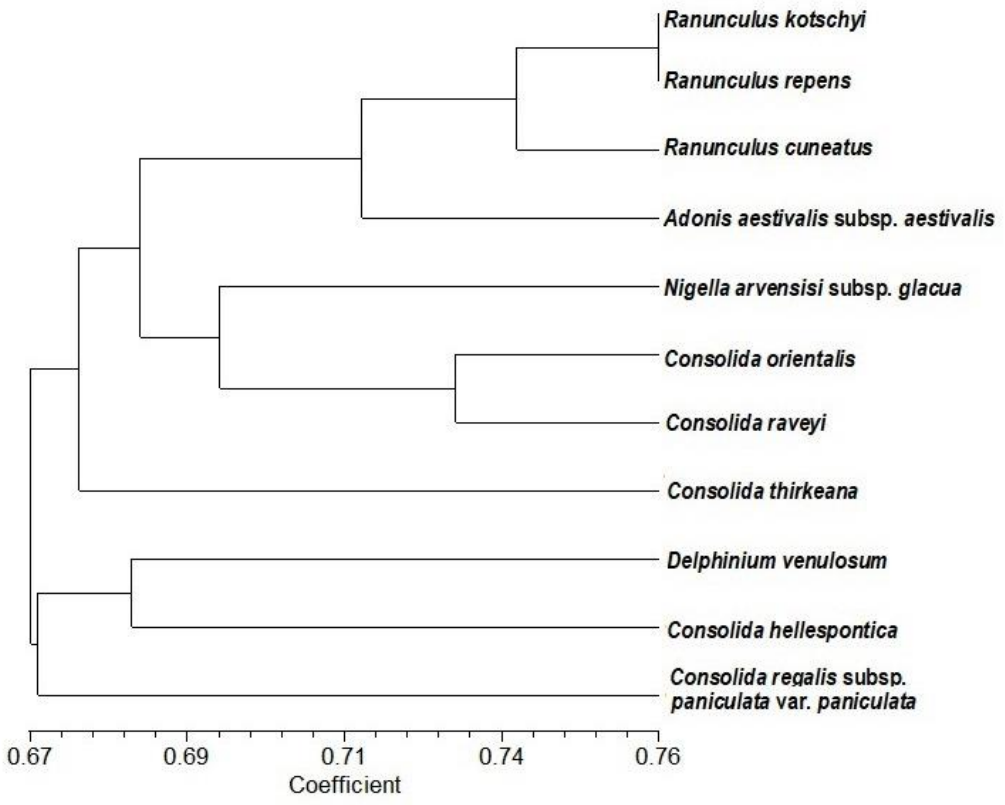

Figure 4.7. The UPGMA dendrogram from the amplified products using iPBS marker system of plant samples from the Ranunculaceae family.

Principal coordinate analysis (PCoA) was conducted to find genetic similarity between species. Similarity index data were analysed via NTSYS program (Rohlf, 1997) utilized to conclude PCoA. Similarity index data were analysed via NT to conclude PCoA and a 3D plot was constructed to show the genetic relationship of species in more detail. The sum of the three dimension of PCoA explained 36.9% of total variation among species (Figure 4.8) Also, this graph clearly discriminated relationship of species on the three dimensional scale similar the dendrogram results.

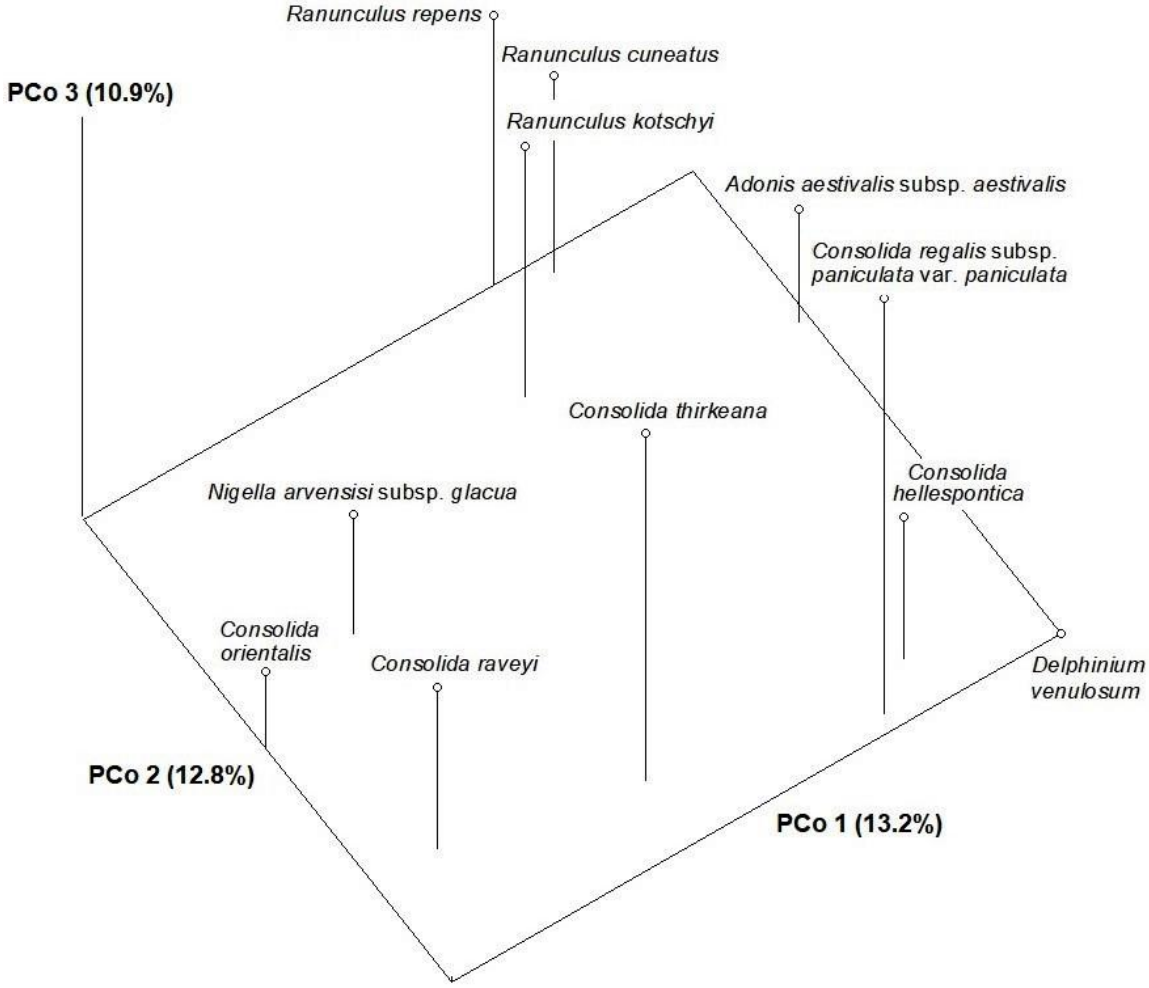

Figure 4.8. The three dimensional view of the amplified products using iPBS marker system after the principle coordinate analysis for the plant samples from Ranunculaceae family.

Similar studies were conducted to investigate the genetic relationship inter-specific or intra-specific, using iPBS molecular markers. One of these studies was performed by Hossein-Pour et al (2019) and the aim was to determine genetic similarities within or between Adonis species. In their study, they worked on 9 species which were collected

from different locations in Turkey using 10 iPBS primers that gave the best results out of 83 iPBS primers. The selected primers produced 206 scorable bands with average of 20.40 per primer. 204 bands were polymorphic among the total bands with average of 20.30 per primer. The maximum scorable bands were obtained by primer 2392 and 2377, on the other hand, primer 2401 gave the less scorable bands. The polymorphism percentage varied from 95% to 100% depending on the primer with an average of 99.5% per primer. The PIC value was calculated per primer which ranged from 0.16 to 0.39 and the average PIC value was found to be 0.30 for each primer. Primer 2385 gave the highest PIC value at 0.39. However, average PIC values were at 0.33 per primer. According to the cluster analysis, the dendrogram demostrated clear distribution between Adonis species. Four main clusters were obtained through UPGMA. PCoA was also performed to discriminate members of Adonis species in more detail. Results stated that the high resolution of genetic relationship was obtained using iPBS marker aproach in terms of Adonis species for breeding programmes. Results of this study were in accordance with Hossein-Pour et al (2019).

Another study was conducted by Bonchev and Ivanova (2020) to determine the genetic variability of some genus of Asteraceae family using the iPBS marker system. This genus included species of *Helianthus, Echinaceae, Tagates, Tithonia* and *Verbesina*. In addition, cultivars which were developed from *Helianthus annuus* cv 1114 (as parent) were selected to determine inheritance of parental genome. For this purpose, primer 2374, 2378, 2222 and 2224 were tested to monitor band profiles which were similar to the parents. Scorable band profiles were obtained with primer 2222 and 2224. The polymorphism percentage varied between 44.8% and 93.3% for primer 2222 and 85.7% and 100% for primer 2224 among studied species. Results indicated that iPBS marker was broadly informative to display genetic relatioship.

The determination of genetic similarities of African *Gnetum* species using iPBS molecular markers was carried out by Doungous et al (2020). *Gnetum* species are used for different purposes in Africa such as medicinal and as a vegetable. Wild populations of Africam *Gnetum* are classified as endangered species. Molecular tools are utilised to better understand new species and their close relatives alongside their taxonomic differentiation. Six accessions were used for both *G. africanum* Welw., and *G. buchholzianum* Engl. In addition, *G. latispicum* a recently identified species had two

accessions in this study. The amplification performance of DNA fingerprinting of 21 iPBS primers were screened; however, only 6 of them gave best band profiles. Therefore, molecular characterization of African Gnetum was carried on with primer 2080, 2081, 2242, 2243, 2270 and 2271. The number of total generated bands were 103 in *G. africanum*, 95 in *G. buchholzianum* and 24 in *G. latispicum*. Polymorphism percentages varied from 90% (2270) to 100% (2271). On the other hand, the polymorphism percentage varied from 95.06% to 100% depending on primers in accordance with our study. Similarity coefficient varied from 0.14 to 0.66 whereas, the similaritiy coefficient ranged from 0.67 to 0.76 for the current studies. Differences in the similarity coefficient and polymorphic values maybe be due to genotype or primer sets which were used for both studies. A dendrogram was generated by cluster analysis which displayed a close relationship between *G. buchholzianum* and *G. latispicum.* PCoA was also performed to discriminate in more detail and explained 55% of variation among the accessions. Also, PCoA analysis clearly discriminated relationship of species on three-dimensional scale similar with the dendrogram. The information which was obtained using iPBS markers indicated that it is useful for the classification, conservation and breeding of African Gnetum species.

Investigation of the genetic diversity contributes useful knowledge to breeding programmes. Yaldız et al (2018) characterized genetic diversity of *Nicotiana tabacum* L. which was collected from different provinces of Turkey. 90 landraces and 6 commercial cultivars were used to assess their genetic relationship. Firstly, the efficiency of 83 iPBS primers were tested on some tobacco landraces. Among the primers, 11 of them gave better results in terms of band profile. 119 bands were obtained as a result of scoring with average of 10.82 per primer. 98 bands were polymorphic among the total bands with average of 8.91 per primer. Whereas, 1420 polymorphic bands was recorded with average of 71 per primer in our study. Primer 2087 produced more scorable bands but 2387 produce less number of scorable bands. On the other hand, Primer 2274, a common primer which was used for our study and in Yaldız et al (2018) produced 87 scorable bands with 100% polymorphism in this present study while it produced 10 scorable bands with 50% polymorphism in the study conducted by Yaldız et al. (2018). Average PIC value was 0.33 per primer in accordance with our result. The 96 accessions were distributed within two main group according to Jaccard's coefficent using R statistical software for analyses. In addition

some accessions were splitted from the two main groups. A dendrogram which was constructed by UPGMA clearly demonstrated two main groups. Results pointed that comparison and evaluation of landraces in consideration with commercial cultivars contribute to the development of new cultivars via breeding programmes.

One of the most important and fundamental steps of plant breeding is the characterization of germplasm. Therefore, Yıldız et al. (2020) focused on characterizing 94 pepper accession consisting 85 in *C. annuum*, 7 in *C. frutescens* and 2 in *C. chinense* and these accessions were collected from different geographical regions. iPBS markers were selected to characterize pepper accessions due to reproducibility, high resolution power and aplicability to all plants. Screening of band profile and polymorphism was done with 20 iPBS primers. All primers succesfully produced 172 scorable bands with average of 8.6 per primer. A total number of polymorphic bands were 158 with average of 7.9 per primer. Although same numbers of iPBS primer were used for our study, the number of total scored bands and polymorphic bands were different with our study. Primer 2272 and 2274 generated 6 bands with 66.7% polymorphism in the pepper study while on the other hand, these primers produced 76 and 87 bands with 98.68% and 100% polymorphism in our study. In addition, primers 2400, 2232 and 2229 were common for both studies. However, these primer sets generated less scorable bands compared to our results. These results indicated that differences of number of total bands and polymorphism percentage may be due to genotypical differences. Average of PIC value was higher (0.66) than our result (0.33). UPGMA, Structure and PCoA were performed to separate groupings. As a result of cluster analysis *C. annum* accessions showed clear ditribution from *C. frutescens* and *C. chinense*. Results explained that geographical origin or fruit characteristic of accessions did not reveal any correlation with iPBS-based clustering.

In recent literature, genetic similarities of potato germplasm has been determined using iPBS marker (Demirel at al., 2018). Firstly, the usefulness of 45 iPBS primers were tested to check the banding pattern on 16 potato germplasm. In the next step, 17 iPBS primers which gave better DNA fingerprinting results were selected to screen genetic relationships between potato germplasms of 76 commercial cultivar and 75 breeding lines. The number of scorable bands were 290 with average of 17.1 per primer. On the other hand, 224 bands were polymorphic with average of 13.2 per primer. However in

our study, 1427 scorable bands were recorded with average of 71.4 per primer and 1420 polymorphic bands with average of 71 per primer. The primer 2229 represented 100% polymorphism in both studies. A formula which was developed by Hinze et al. (2015) was used to calculate the PIC value. According to the formula, PIC value was ranged between 0.12 to 0.31. The maximum and minimum PIC values varied between 0.12 (2375) to 0.31 (2229). According to our result, PIC values ranged from 0.29 (2274) to 0.39 (2402). Clear distribution of germplasm was performed by Jaccard's coefficient analysed by PAST 3.14 software to find the genetic similarity via neighbour- joining methods. Potato germplasms were distributed within nine groups which were also separated into subgroups. Results emphasized that knowledge of iPBS markers was helpful to indicate genetic closeness and selection of parental individuals for developing candidate cultivars for plant scientist.

Karık et al. (2019) assessed the genetic relatedness of 94 Laurel genotypes using iPBS molecular tool. These genotypes were collected from different regions of Turkey. Determination of the efficiency of iPBS primers were performed with eight laurel genotypes. 13 primers having polymorphic features were choosen to investigate the genetic similarity. These primer sets produced 159 bands with average of 15 per primer. The total number of polymorphic bands was 164 with average of 12.6 per primer. The number of polymorphic bands is 1420 with average of 71 per primer according to our results. The maximum polymorphism percentage was given by primer 2402 in our study.

CHAPTER V

CONCLUSIONS

The study on the morphological, physiological, and molecular characteristics of wild flower species of the Aladağ mountains with special horticultural landscape properties, revealed that, *C. orientalis* and *C. raveyi* require cold stratification to improve germination percentages under laboratory conditions at a temperature of 20°C. The most effective duration of stratification for *C. raveyi* seeds was the 15 days period whereas that for *C. orientalis* was 45 days.

PGR priming was carried out on *C. orientalis* and *C. raveyi* seeds storing them under two conditions, of 4°C and at room temperature. Seven different concentrations (5, 15, 30, 50, 100, 300 and 500 ppm) of GA_3, IBA and kinetin, plant growth regulators, were used as priming agents for the storage of seeds under both conditions. The results for *C. orientalis* indicate that the seeds treated with GA_3 at concentration 300 ppm saw increase in the germination percentage (35.7) for seeds stored at 4°C. However, at other concentrations, there was a decline in the seed germination percentage. The same trend was observed in seeds stored at room temperature. The *C. orientalis* seeds stored at 4 °C germination percentage decreased after treatment with different concentrations of IBA however application of 500 ppm IBA for seeds stored at room temperature increased seed germination percentage (30.3%). In the kinetin treatments, germination percentages declined in treatments at all concentration levels for seeds stored at 4°C while an increase was observed at 5 ppm for seeds stored at room temperature. The optimum temperature for germination was 10°C under both storage conditions for all treatments, with no seed germination observed at 20°C.

For *C. raveyi* seeds, the application of GA_3 at the concentration of 500 ppm resulted in increased germination percentage for seeds stored at 4°C but there was no significant effect on seeds stored at room temperature at all applied concerntrations. For treatment with IBA, maximum germination percentage was observed for seeds stored at 4°C at concerntration 100 ppm and minimal percentage at 30 ppm while those at room temperature had their highest at concerntration 300 ppm and lowest at 15 ppm. Under kinetin treatments, the highest germination percentages were observed in the control

group rather than under the storage conditions indicating that the treatment have no significant effect on germination percentage. However, under both storage conditions, maximum germination percentage was observed at 10°C in all three PGR treatments. Analyzing the study critically, one can conclude that, both species thrive better under low temperature conditions as far as improved germination percentages are concerned.

Species under this study were well adapted under greenhouse conditions. These species can be used as ormanamental plants in future.

The molecular analysis of 11 plant species from the Ranunculaceae family using iPBS markers produced between 50 and 91 scorable while polymorphism percentage varied from 95.06% to 100% depending on the primer with an average of 99.6% per primer depicting the relatedness of species in the family. The calculated PIC value was from 0.29 to 0.39 per primer with an average of 0.33 for each primer. Primer 2402 gave the highest PIC value of 0.39.

The high polymorphism rates and the high number of bands recorded using a single primer shows that the iPBS marker is informative which can be used for a comprehensive study on populations, taxonomy, conservation and domestication of members of the Ranunculaceae family. These results further reveal the diversity and evolution of related species and their usefulness in breeding.

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