ADIL RIAZ

ENHANCING THE REPRODUCTION OF *Tulipa cypria* BY SEEDS

MASTER THESIS

2024

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF LANDSCAPE ARCHITECTURE

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M.Sc. THESIS

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M.Sc. THESIS

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January, 2024

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Declaration

I hereby declare that all information, documents, analysis, and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

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20/01/2024

Acknowledgements

I would like to express my deepest appreciation to my Supervisor Assoc. Prof. Dr. Salih Gucel for his guidance and without his supervision my thesis work could not have been possible and for shaping my colloquial opinionated writing into something that resembles academic research writing.

My special thanks to my co-supervisor Assoc. Prof. Dr. Özge Özden, for her support and encouragement as I wrote my thesis and for shining a bright light on it. Her support inspired me to work hard and enthusiastically finish my thesis. All thanks to the Near East University and the Department of Landscape Architecture.

Special thanks to my family and my friends for providing moral and emotional support without them this thesis couldn't have been complete.

Adil RIAZ

Abstract

ENHANCING THE REPRODUCTION OF *Tulipa cypria* BY SEEDS

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January 2024 pages 49

Tulips are important bulbous and ornamental plants used as cut flowers. Among the tulip species, *Tulipa cypria* is a species that is found on the island of Cyprus. *T. cypria* is classified as "Endangered B2ab(iii)" on the IUCN Red List of Threatened Species (2011) and is protected by both local and international regulations. It is also protected by the Protection of Northern Cyprus Flora and Fauna Ordinance (Ordinance 21/97 Environment Law 10 (2), and cutting *T. cypria* blooms or taking wild bulbs is illegal. Despite all these conservational techniques, it is still threatened by animal grazing and forest fires and a low germination rate. To enhance its reproduction, we applied some seed dormancy-breaking techniques. This experiment was conducted in 2023 at Near East University Lefkosa, TRNC. In which we used stratification of seeds at 4°C, using gibberellic acid with 100, 300, and 500 ppm concentration and potassium nitrate with 0.1%, 0.2%, and 0.3% concentration. The germination percentage and Mean germination time of the seed were observed in this experiment. In this study, we found that stratification at 4°C gave the best result of seed germination and also took less time for germination. The seed treated with gibberellic acid and potassium nitrate also enhances the (FGP)and (MGT) of seeds.

***Key words*:** Cyprus; endemic species; seed dormancy; stratification; *T. cypria*

Özet

TOHUMADM *Tulipa cypria* ÜRETİMİNİN ARTIRILMASI

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Ocak 2024, 49 sayfalar

Laleler, kesme çiçek olarak kullanılan, önemli soğanlı ve süs bitkilerindendir. Lale türleri arasında Tulipa cypria, Kıbrıs adasında bulunan bir türdür. T. cypria, IUCN Tehdit Altındaki Türler Kırmızı Listesi'nde (2011) "Tehlike Altındaki B2ab(iii)" olarak sınıflandırılmıştır ve hem yerel hem de uluslararası düzenlemelerle korunmaktadır. Ayrıca Kuzey Kıbrıs Flora ve Faunasının Korunması Yönetmeliği (Yönetmelik 21/97 Çevre Kanunu 10 (2) ile koruma altına alınmış olup, T. cypria çiçeklerinin kesilmesi veya soğanlarının alınması yasaktır. Tüm bu koruma tekniklerine rağmen, hayvan otlatma, orman yangınları ve çimlenme oranının düşük olması nedeni ile hala tehdit altındadır. Üremesini arttırmak, için bazı tohum uyku hali kırma teknikleri uyguladık. Bu deney 2023 yılında Yakın Doğu Üniversitesi Lefkoşa, KKTC'de gerçekleştirildi. Tohumların 4OC'de soğuklsnması yanında, 100, 300 ve 500 ppm konsantrasyonlu giberellik asit ve %0,1, %0,2 ve %0,3 konsantrasyonlu potasyum nitrat kullanılarak yapılan bu deneyde tohumun çimlenme yüzdesi ve ortalama çimlenme süresi gözlemlendi. 4°C'de yapılan soğuklamanın tohum çimlenmesinde en iyi sonucu verdiğini ve çimlenmenin daha az zaman aldığını, Gibberellik asit ve potasyum nitratla muamele edilen tohumun, tohumların nihai çimlenme yüzdesini (FGP) ve ortalama çimlenme süresini (MGT) artırdığını ortaya koydu.

***Anahtar Kelimeler*:** Kıbrıs; endemik türler; tohum dinlenmesi; tabakalaşma; *T. Cypria*

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List of Abbreviations

TRNC	Turkish Republic of Northern Cyprus
NEU	Near East University
FGP	Final Germination Percentage
MGT	Mean germination time
IUCN	International Union for Conservation of Nature

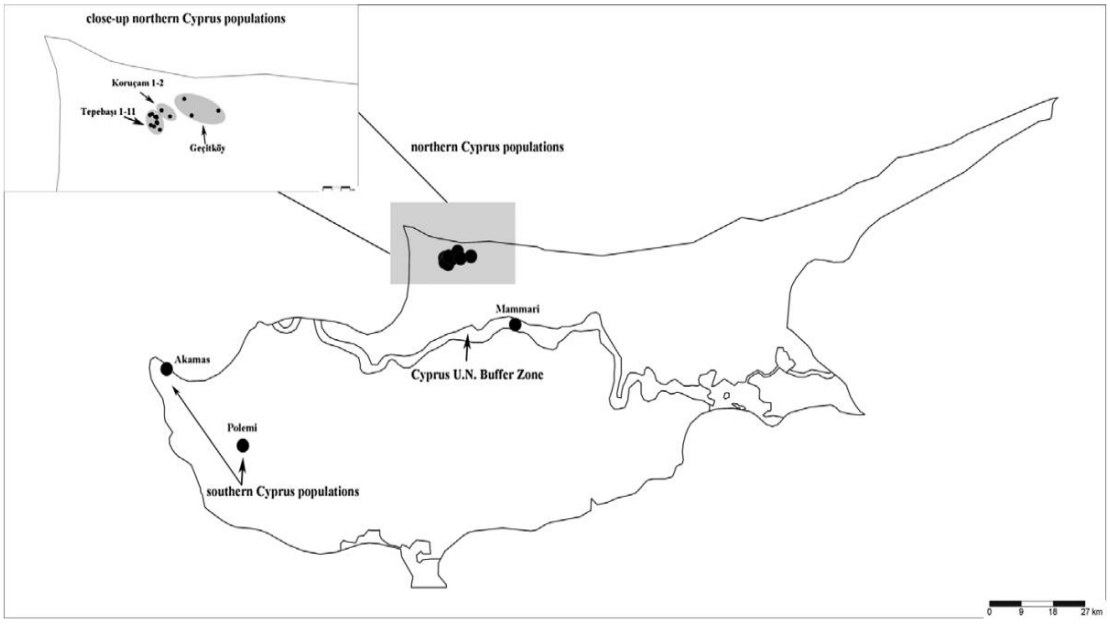
CHAPTER I

Introduction

Tulip hybrids are important ornamental plants used for cut flowers because they are produced and traded on a global basis. Botanical tulips are almost as well-known as decorative plants (Pipinis et al., 2023). Tulip is a perennial decorative crop of the Liliaceae family, native to Central Asia, the Mediterranean, Europe, and North Africa, with roughly 139 species worldwide. It is a valuable decorative bulbous plant with a wide range of bloom hues that can be used as cut flowers, potted plants, and garden plants for landscaping. (Xing et al., 2017). Among 139 species of the genus Tulipa, *T. cypria* represents an endangered local endemic species of the island of Cyprus in the Mediterranean region. *T. cypria* is located in the villages of Geçitköy, Koruçam, Tepebaşi (Kormakiti-Myrtou-Panagra), Mammari, and the Akamas. Its natural habitats include Maquis, Juniperus phoenicea L., pastures, and wheat fields on limestone at elevations ranging from 100 to 300 meters (Tsintides et al., 2007).

Figure 1.1

Showing the distribution area of T. cypria



(Trias-Blasi et al., 2017)

T. cypria is classified as "Endangered B2ab(iii)" on the IUCN Red List of Threatened Species (2011) and is protected by both local and international regulations. It is an Annex II plant species under the EU Habitats Directive (92/43/EEC) and grows in the Akamas range, a state forest and Natura 2000 site (Tsintides et al. 2007). It is also protected by the Protection of Northern Cyprus Flora and Fauna Ordinance (Ordinance 21/97 Environment Law 10 (2), and it is illegal to cut *T. cypria* blooms or take wild bulbs. Due to all these conservation techniques, this species is still threatened by overgrazing by animal grazing, livestock farming, expansion of housing and urban areas, and forest fires. If the species is to survive, its conservation status must be urgently re-evaluated and solutions undertaken (Bilz, 2013). In this study, we aim to effectively enhance reproduction from seeds of this species by applying some dormancy-breaking techniques to conserve this endangered species.

Background of the Study

T. cypria is indigenous to Cyprus, where it can be found in three places across a 44-kilometer region. The population has been estimated to number over 6,000 individuals, with an unclear trend. The International Union for Conservation of Nature (IUCN) states that it is classified as an endangered species. Animal grazing is a threat to the *T. cypria* species. Harvesting of this flower, urbanization of the areas where these flowers grow, use of herbicides on crops, and Wildfires in the forest are an important factor affecting the population of *T. cypria.* (Bilz, 2013). According to Tsintides., et al (2007), this species is threatened by overgrazing, overcollection, urbanization, and inadequate regrowth. It can be located in three different locations on the island of Cyprus. The Akamas woodland, Kormakiti-Myrtou-Managra, and Mammari are between 100 and 300 m altitude. It grows in maquis, pastures, and limestone cereal fields of *Juniperus phoenica* L. Furthermore, the Northern Cyprus Flora and Fauna Protection Ordinance (Ordinance 21/97 Environment Law 10 (2)) protects it, therefore it is illegal to destroy *T. cypria* blooms or take wild bulbs.

Significance of Study

T. cypria is an endangered and threatened species by different environmental factors. The conservation status of the species needs urgent re-evaluation and strategies for its survival. The germination rate of this species is very inadequate.

Purpose of the Research

The germination rates of tulip species are so adequate that they need proper treatment for their germination. For this purpose, some techniques are used to enhance germination by breaking the dormancy of seeds of *T. cypria*. This technique will help to re-evaluate and enhance the reproduction of this threatened species. The objectives of this research are given below:

- ✓ To enhance the reproduction of *T. cypria*
- ✓ To check the effect of different treatments on breaking the dormancy of seeds.
- ✓ To enrich the wild population by planting bulbs in the wild.

Limitations of the Study

This study was only limited to three treatments Stratification, Giberallic acid solution and Potassium nitrate solution each having three replications for *T. cypria.*

CHAPTER II

Literature Review

Tulip

The Ottoman Empire introduced ornamental Tulips to Istanbul in the sixteenth century. The tulip custom was most likely brought with the Seljuks on their journey from Central Asia to Anatolia. The tulip trend that swept the Ottoman world in the sixteenth century was shortly to travel westward to Europe. The Flemish Ogier Ghiselin de Busbecq (1522-1592), the Habsburg emperor's ambassador in the Ottoman court in Istanbul in 1554-1562, was long regarded as the first to introduce tulips to Europe (Stefanaki, 2022). There are about 13,000 acres worldwide dedicated to the production of tulip bulbs (Orlikowska, 2018). The Liliaceae family includes approximately 139 species that constitute the genus Tulipa. The species is indigenous to southern Europe, northern Africa, and Asia, as well as western and northeast China. The Pamir and Hindu Kush mountains, as well as the Kazakhstani plains, are where the genus is mainly encountered (Rouhi et al., 2012).

By taxonomic classification, (Van Raamsdonk & De Vries, 1995) divided the genus Tulipa into the subgenera Tulipa and Eriostemones. The World Checklist of Selected Plant Families lists 554 taxa for the genus Tulipa (Orlikowska, 2018). Tulips come in a variety of species and hybrid varieties that be grown in gardens, potted, or used as fresh-cut flowers (Rouhi et al., 2012). The indigenous tulip *T. cypria* is one of the most distinctive plant species. Based on physical similarities and sympatric distribution in Cyprus, *T. cypria* has been proposed as a mutant of *T. agenensis*. (Wilford, 2006; Christenhusz et al., 2013).

Figure 2.1

(A) *T. cypria* seeds (B) seeds pod (C) *T. cypria* flower

(A) (B) (C)

(Mifsud, 2022)

Table 2.1

Scientific classification of T. cypria

Kingdom	Plantae
Order	Liliales
Family	Liliaceae
Genus	*Tulipa*
Species	*T.cypria*

It has a single, unbranched stem that extends between 15 and 40 cm from the ground with a single flower, and a lengthy corm that is frequently buried underneath. The two lowest leaves are the largest, measuring up to 4 cm broad and 30 cm in length. The flower blooms during March and April and has a dark crimson hue with a small, sometimes absent, rounded black spot inside at the base that is normally flanked by an extremely short yellowish area (Meikle, 1985; Trias-Blasi et al., 2017). The viability and reproduction of seeds in their natural habitat are guaranteed by their inbuilt physiological dormancy. Yet, the development of one's population and corporate

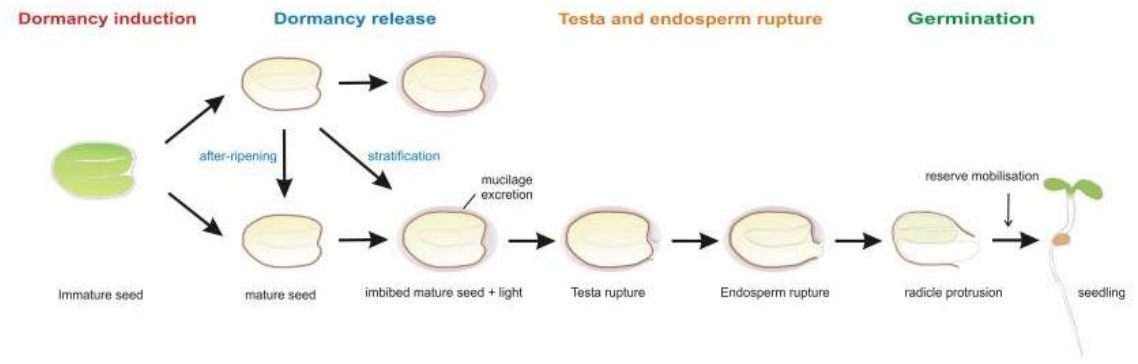
breeding are hampered by the minimal germination rate. Seed dormancy is controlled by a variety of circumstances. Temperature is a key environmental component that affects how seeds modify their dormancy, variable temperatures, and stratification can break dormancy. Temperature variations have often been found to encourage seed germination further than stable temperature (Rhie & Lee, 2020; Loddo et al., 2019).

Seed Dormancy

A process under which seeds delay the germination of an undamaged, viable seed and wait for better conditions to germinate is known as seed dormancy (Finkelstein et al., 2008). The effects of abscisic acid during seed development are really what induce primary dormancy. However, normal dormancy is also referred to as secondary dormancy. Numerous species frequently experience seed dormancy. It is adaptation that enables a species to control when seeds will germinate. Several species' germination time of seeds according to external factors like temperature and rainfall etc. Some species have evolved to allow for asynchronous germination for more than a long period. As a result, a stable seed bank can be established and frequent germination is made possible (GASHI et al., 2019).

Figure 2.2

Schematic presentation of seed dormancy



(Bentsink & Koornneef, 2008)

Seed dormancy has been reduced or eliminated because of crop plant adaptation to coincide with the growing season. Even though the majority of the important flower species still express some sort of dormancy in their seeds, this harms seed yield and

makes seed germination more difficult. Primary and secondary dormancy are the two main types of dormancies. The primary dormancy is split into three groups. Exogenous, endogenous, and combinational dormancy are among them (Hartmann et al., 1997; Geneve, 1998). Entities outside the embryonic part induce exogenous dormancy some examples include Maternal tissues, seed coats, or carp. The embryo's internal dormancy components are linked to endogenous dormancy. Exogenous and/or endogenous dormancy are both included within the combinational dormancy. When the germination environment is Undesirable, some non-dormant seeds are placed under a secondary dormancy (Geneve, 1998). Dormancy can be re-induced when an imbibed non-dormant seed is subjected to specific sustained adverse conditions. Secondary dormancy refers to re-induced hibernation. Thus, secondary dormancy is not produced during seed maturity, and induction does not occur. Secondary dormancy occurs in imbibed ripe seeds rather than dry seeds (Buijs, 2020).

Historical Perspective Regarding Seed Dormancy Techniques

Dormancy has historically been connected adversely with a growing period in which a viable seed does not germinate despite appearing to have adequate environmental conditions. The time between seed dispersal and germination is delayed by all forms of dormancy, however, the root causes can differ (Finch-Savage & Leubner-Metzger, 2006; Rouhi et al., 2012).

Type of seed dormancy brought on by both environmental and genetic variables is a characteristic known as hard seedness, which is common in several species of plant families including Leguminousae, Malvaceae, and Liliaceae (Copeland and McDonald, 2001; Rouhi et al., 2012).

The breakdown of the seed coat known as scarification, a period of dry storage, stratification, exposure to light, or GA_3 all have been shown to negatively impact the ability of seeds to emerge from such a delayed state of seed dormancy (Finkelstein et al., 2008). Even while germination is a customizable occurrence for every seed, populations exhibit varying degrees of dormancy, which is represented in the germination percentage under particular circumstances (Rouhi et al., 2012).

A tiny subset of the 136 tetracyclic diterpene gibberellins, including gibberellic acid (GA), are functional as plant hormones and promote germination of seeds in several plant species; the majority of active GA varies depending on the species (Thomas et al., 2005). Germination is promoted by gibberellins, by activating hydrolytic enzymes that degrade barrier tissue (endosperm or seed coat), releasing seed storage reserves, and promoting embryo growth (Bewley & Black, 1994).

Stratification is the process of keeping seeds in moist circumstances to overcome dormancy, typically in a cold environment to imitate overwintering (Finkelstein et al., 2008). Utilizing stratification treatment, the impact of GA_3 has reportedly been found to promote germination rate (Yamauchi et al., 2004). Stratification is crucial in delivering the stimuli needed to break out dormancy. It has been noted that stratification triggers a rise in GA_3 production. (Bretzloff and Pellett, 1979; Yamauchi et al., 2004; Rouhi et al., 2012).

Nitrogen oxide gas, nitrite, nitrate, and several other nitrogen compounds, encourage the breaking of dormancy and seed germination in a variety of species, potentially as a way to detect the presence of nitrogen in soil (Bethke et al., 2007). It is well understood that the chemical potassium nitrate promotes photo-dormant seed germination (Shanmugavalli et al., 2007). To interrupt seed dormancy and improve plant health, several gardeners adopt potassium nitrate. KNO_3 raises atmospheric oxygen concentration by reducing the oxygen supply for citric acid processes (Bewley & Black, 1994). When the seed coat is damaged or removed, dormant seeds that express seed-covering dormancy will reactivate. For breaking dormancy in certain species, scarification using sulfuric acid is necessary (Finkelstein et al., 2008; Rouhi et al., 2012).

According to Sixtus et al. (2003) hot water treatment did not affect the germination of Ulex europaeus seeds, but the treatment by sandpaper and sulfuric acid effect well. Parkia biglobosa seed dormancy breakdown was reportedly affected through the application of sulfuric acid, hot water, and scarification by sand paper (Rouhi et al., 2012).

Current Seed Dormancy Treatments

According to past reviews, seed dormancy is among the processes in seed physiology that is least known (Finch-Savage & Leubner-Metzger, 2006). This may be because various plant species exhibit and break dormancy in various ways. The misunderstanding explanation of seed dormancy was previously described in ecological and physiological investigations; however, it is now understood that dormancy is indeed a state with numerous causal factors rather than a single factor (Miransari & Smith, 2014).

Modifications at the cellular level, such as protein and hormone variations, and the equilibrium among ABA and gibberellins are some of the most crucial factors that impact the procedure of seed dormancy (Finch-Savage & Leubner-Metzger, 2006) (Finkelstein et al., 2008; Miransari & Smith, 2014). Certain environmental elements, including light, hormonal therapy, freezing, and hormone levels, can induce germination in the seeds of a relatively small number of species, by breaking the seed dormancy (Miransari & Smith, 2014).

Although they seem to have no role in controlling seed dormancy, gibberellins are crucial for promoting and enhancing germination in addition to activating dormant seed (Miransari & Smith, 2014). As ABA can hinder the germination of seeds in some plant species, whereas increased GA concentration either increases germination rate or is essential for seed germination, it is believed that ABA and GA function are closely connected (Bewley, 1997; Miransari & Smith, 2014; Zhang et al., 2020).

To promote the germination of seeds and disrupt seed dormancy, gibberellins and nitrate are applied. Similar to a supply of nitrogen, NO_3 promotes germination. By boosting amylase activity, changing the K+/Na+ ratio, enhancing ATP generation, and reducing the amount of ABA in the seed, N molecules can suppress seed dormancy and have an important impact on germination (Alboresi et al., 2005). Researchers have discovered that NO_3, like KNO_3, can promote the regeneration of dormant seeds (Alboresi et al., 2005; Zhang et al., 2020).

Treatments of 0.1 to 0.2% KNO_3 are frequently used in regular germination tests and are advised for germination tests of many species, as recommended by the

Association of Official Seed Analysts and the International Seed Testing Association (ISTA, 1996). Nevertheless, plant hormones like gibberellins and cytokinin can negatively react with ABA to effectively influence the processes of germination percentage (Hermann et al., 2007). The activation of catabolizing enzymes (nitrite and nitrate reductase and glutamine synthetase) and suppression of the ABA biosynthetic pathway are two biochemical processes that are known to be increased by GA. Furthermore, GA promotes the germination of seeds by producing an amylase (Finch-Savage & Leubner-Metzger, 2006; Zhang et al., 2020).

Treatments to Lift Seed Dormancy

Temperature Control

Many studies have demonstrated that the seeds of tulip species require a long time of low temperature and high humidity to germinate. Nabieva and Gerasimovich discovered that when isolated *T. kaufmanniana* embryos were not frozen, no adventitious bulbs formed, confirming that low temperature (4 °C) played a crucial role in seed germination (Yurievna & Vladimirovna, 2020). The mean temperature in early spring (January to March) was 4 °C in the Tianshan Mountains of Xinjiang, China, with the maximum daytime temperature approaching approximately 16 °C.

In a study on *T. thianschanica*, the highest seed germination percentage was seen at a constant temperature of 4°C and/or a variable temperature regulation of 4/16°C. According to research, seeds respond to treatments at varying temperatures. Increased temperature prevented breaking of dormancy and/or germination, while only a few seeds germinated at 16°C and 16/20°C, indicating that 16°C may operate as a threshold level for germination (Zhang et al., 2020).

Phytohormones Promote the Seed Germination

Gibberellin and abscisic acid, in particular, were phytohormones involved in germination percentage, and it was discovered that their combination had considerable detrimental consequences on seed germination (Zhang et al., 2020).

Cold temperatures and GA_3 exposure were able to break the profound dormancy of wild *Tulipa gesneriana* seed. To boost sensitivity, avoid dormancy, and respond better to GA_3 dosage, stratification is crucial (Finkelstein et al., 2008). Pre-chilling seeds

at the right time and for the right amount of time encourages germination (Nkomo & Kambizi, 2009). As GA_3 activates hydrolytic enzymes to break down biological dormancy in seeds having dormant embryos, its physical significance as a germination booster in the germination of dormant seeds in a variety of plant species has long been understood (Miransari & Smith, 2014). By stimulating the creation of proteins as well as other essential metabolites for the embryos, GA_3 enhances seed germination (GASHI et al., 2019).

Gibberellin, which allows seeds to emerge from dormancy, was crucial in fostering testa to rupture (Liu et al., 2016). Plantago ovate, Rudbeckia hirta, and Satureja hortensis all showed considerably higher seed germination rates when GA_3 was used. The seeds of Tulipa (*Tulipa Iliensis* and Tulipa Sinkiangensis) also germinated faster when GA_3 was added Nitrate, Nitrite, and cyanide are examples of nitrogenous substances that positively affect the breaking of dormancy. Instead of being a nutrition that decreased the amount of dormancy, nitrate operated like a signal-regulating chemical. Nitrate altered seed dormancy by inducing the CYP707A2 gene transcription during seed development and germination Insignificant but to some degree, KNO_3 significantly enhanced germination of seeds (Zhang et al., 2020).

Impact of Cold Stratification Regulation

Seed dormancy might be reduced effectively with cold stratification (Jan et al., 2013). Regarding *Tulipa kaufmanniana* Regel, Rouhi (2010) concluded that stratification over 49 days was much more successful compared to 35 day. Furthermore, studies found that seeds that had been cold-stratified for less than 15 days may partially germinate, whereas seeds that had been cold-stratified for 25 days could almost entirely germinate. According to this, Tang observed that *T. ıliensis* and *Tulipa sinkiangensi* seeds required cold stratification of more than 4 weeks to emerge from dormancy.

'Negrita' and *T. Thianschanica* hybrid F1 seeds need 36-67 days of cool stratification (4°C) to germinate. These findings suggested that *T. Thianschanica* seeds remained in the soil for an extended period during the warm summer and thrived under favorable conditions in early spring.

CHAPTER III

Material and Methods

The experiment was carried out at the laboratory of the Faculty of Agriculture, Department of Landscape Architecture, Near East University (NEU), Turkish Republic of Northern Cyprus (TRNC).

T. cypria seeds were obtained from Tepebaşı and the surrounding territory during the summer of 2023. Seeds were extracted from fully ripe pods

Figure 3.1

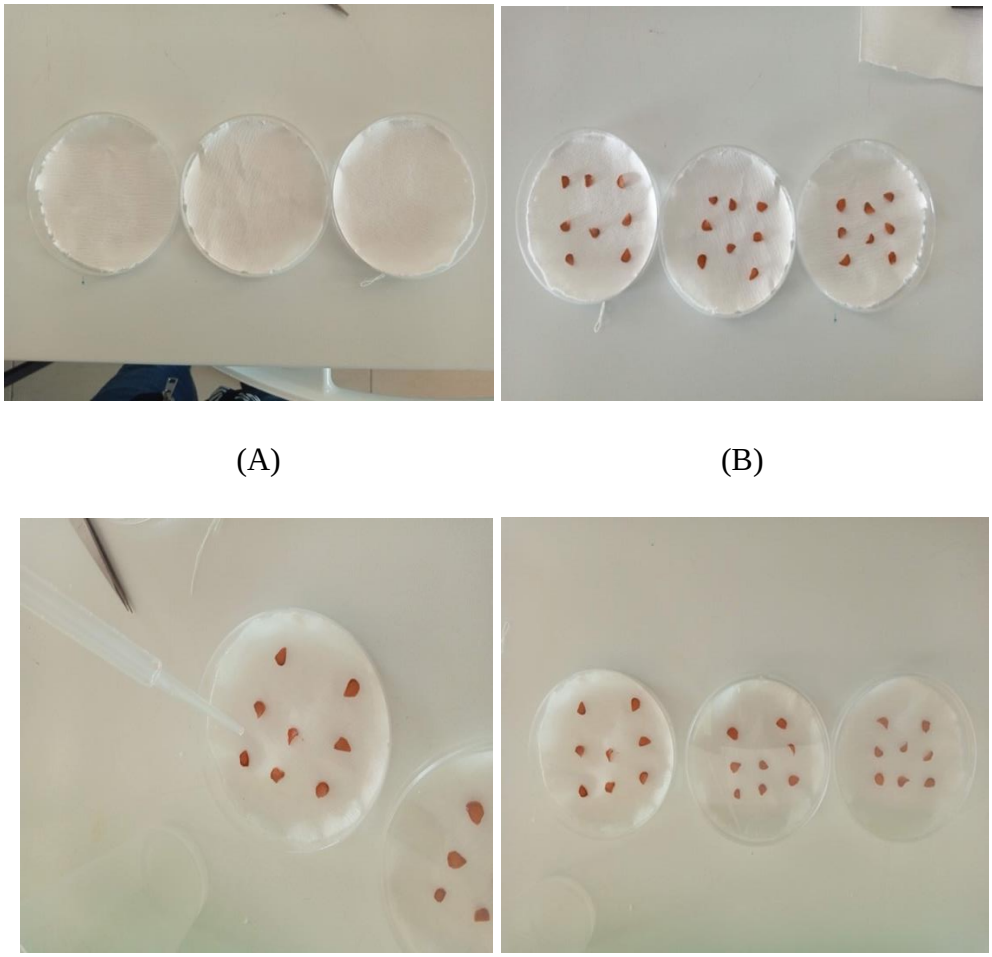
Seeds of T. cypria

Stratification Treatment

T. cypria seeds were put in a petri dish for stratification treatment. To prevent moisture loss, the seeds were placed on a moist paper towel and the petri dish was sealed with stretch film. The petri dish was then placed in a refrigerator at 4°C for 3, 5, and 7 weeks.

Figure 3.2

Stratification treatment of seeds. Placing of paper towel and seeds in a petri dish. (A), (B) Moistening of seeds and sealing the petri dishes (C), (D).



(A) (B)

(C) (D)

Gibberellic Acid Solution

Gibberellic acid solution is prepared for the treatment of seeds. Three concentrations of 100, 300, and 500 ppm solution were prepared. To prepare a 100-ppm solution of gibberellic acid solution 100ml of gibberellic acid is added to 900ml of water to get 100ppm of solution of gibberellic acid. To prepare a 300-ppm solution of gibberellic acid, 300ml of gibberellic acid is added to 700ml of water to get 300 ppm of the solution. To get 500 ppm of Gibberellic acid solution add 500ml of gibberellic acid into 500ml of water to get 500 ppm of solution.

Potassium Nitrate Solution

The solution of potassium nitrate with concentrations of 0.1%, 0.2%, and 0.3% is prepared for the treatment of seeds. To prepare this solution of 0.1% 10.11 grams of potassium nitrate is added to distilled water. Once the KNO_3 is fully dissolved, add more distilled water to bring the volume up to the 1-litre mark on the flask. To prepare 0.2% of the solution add 20.22 grams of potassium nitrate in distilled water and to prepare 0.3% add 30.33 grams of potassium nitrate in distilled water.

Seed Treatment

Seeds of *T. cypria* were surface sterilized with 70% of ethanol for 3 minutes to avoid any fungus attack and after that rinsed with water 3 times.

Figure 3.3

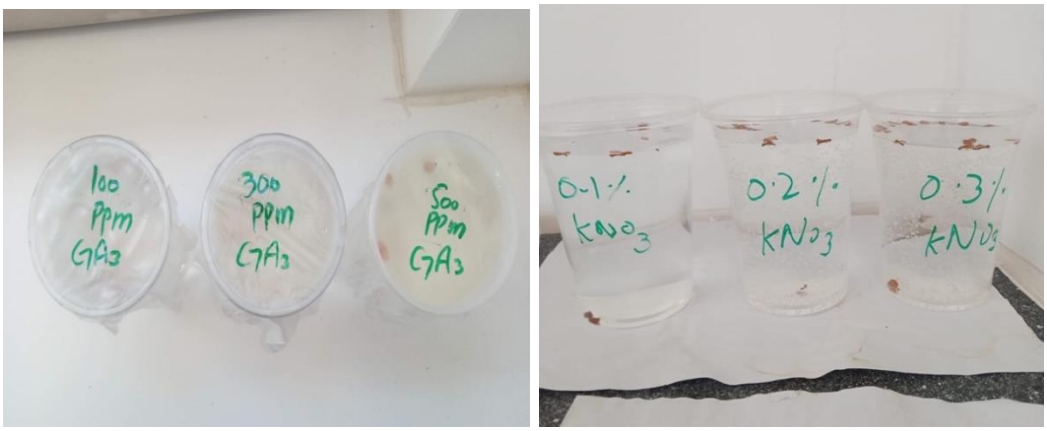
Seed treated with 70% Ethanol

Priming

After the treatment the seeds were soaked in 100, 300, and 500 ppm and 0.1%, 0.2% solution of gibberellic acid and potassium nitrate for 24 hours. For the priming of seeds with potassium nitrate solution, the seeds were soaked for 24 hours.

Figure 3. 4

Priming of the Seeds. Giberallic Acid solution (A). *Potassium nitrate solution* (B).



(A) (B)

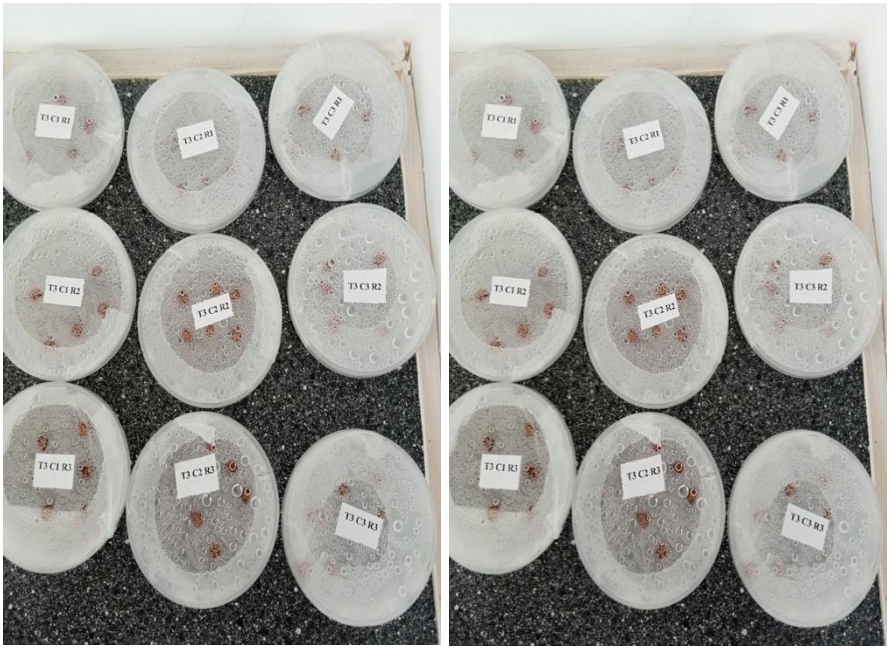
Planting

After 24 hours of priming the seeds were placed in a double-layered paper towel in a 9cm petri dish evenly distributed and moistened with water after moistening the Petri dishes were sealed to avoid moisture loss and placed for germination at room temperature.

Germinated seeds were recorded daily until the end of the experiment. Seeds were considered to have germinated when the emerging radicle elongated to 2mm. germination experiments were set according to a completely randomized design with three replicates.

Figure 3.5

Planting of Seeds in a Petri dish. Seeds treated with GA_3 (A). *Seeds treated with KNO_3* (B).



(A) (B)

In this study, the following parameters were measured.

1. Germination rate (percentage)= Number of germinated seeds/Number of total seeds X 100.
2. Mean germination time=ΣDn/Σn

Where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of germination.

Statistical Analysis

The statistical analyses were of a completely randomized design. Three replications and 6 seeds per replication were used. The mean was calculated using statistics 8.10 and the mean will be compared by tukeys HSD test (5%)

CHAPTER IV

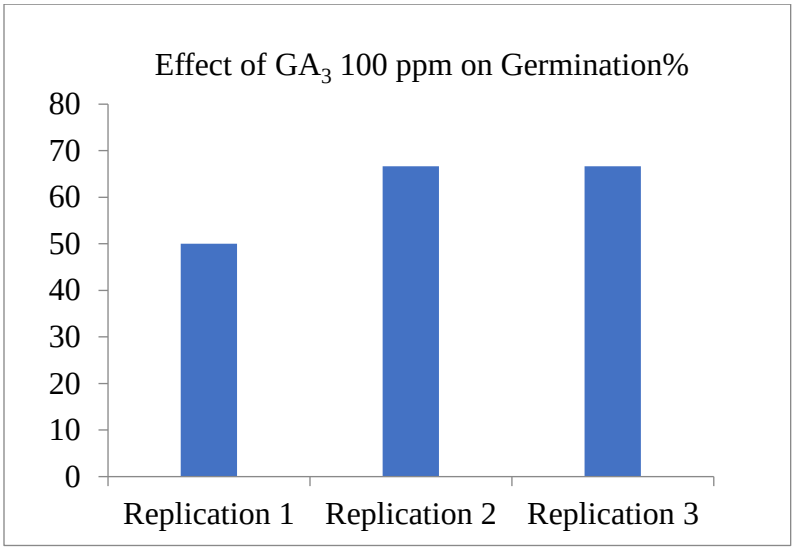
Results and Discussions

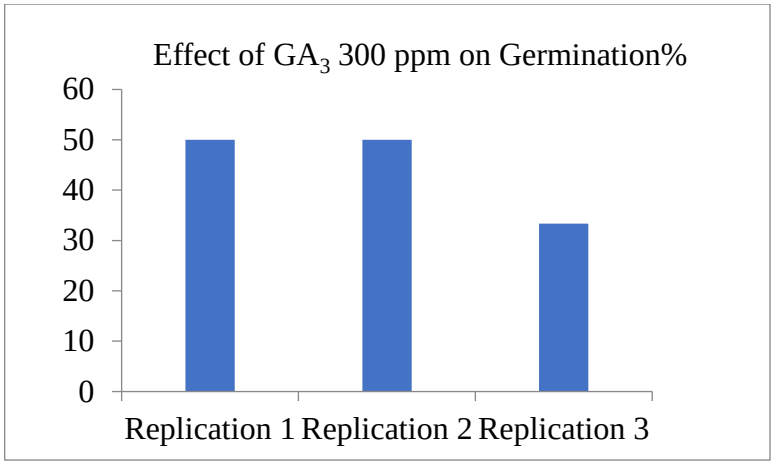
Effect of Gibberellic Acid on Seed Germination

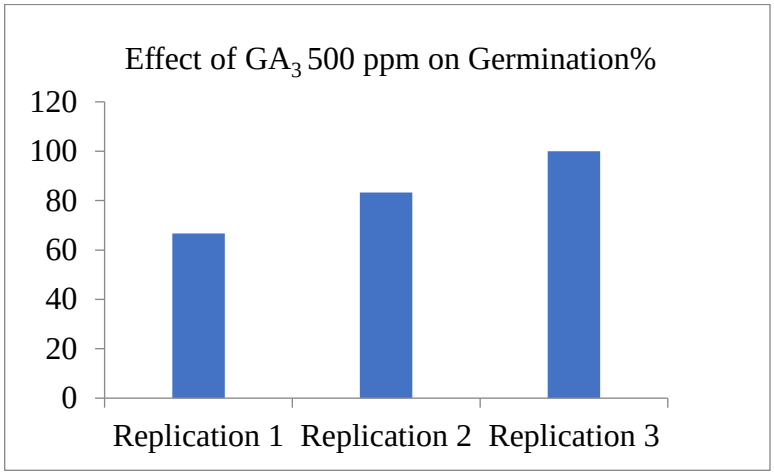
To improve seed germination, the plant growth regulator GA_3 was also used. The use of GA_3 in *T. cypria* seeds has a substantial influence on FGP. The GA_3 plant regulator was utilized to promote *T. cypria* germination. As illustrated in Figure 4.1 three concentrations of GA_3 were utilized to boost germination. The GA_3 concentrations of 500 ppm result in satisfactory seed germination. In contrast to the current study's findings, germination of *T. cypria* seeds treated with 500 ppm of solution is 83%. The germination rate of *T. cypria* seeds is increased when the concentration of GA_3 is increased. Germination increases with increasing GA_3 concentration, as indicated in the figure.

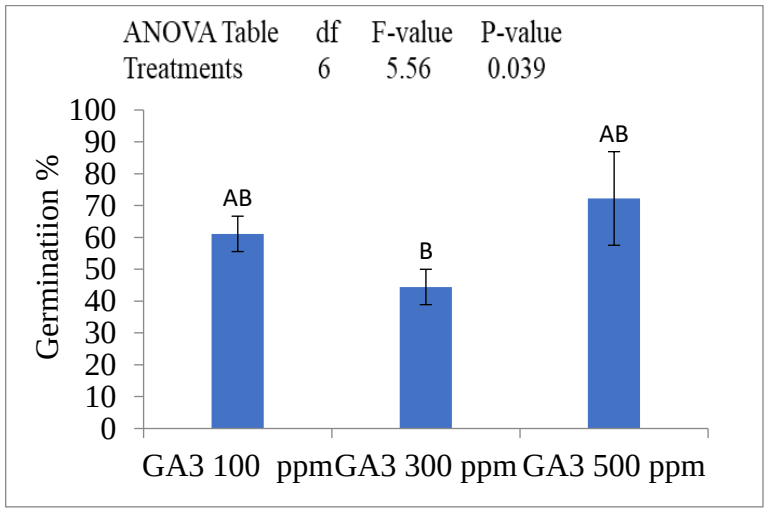
Figure 4.1

The Germination% with GA_3 Giberallic acid effect









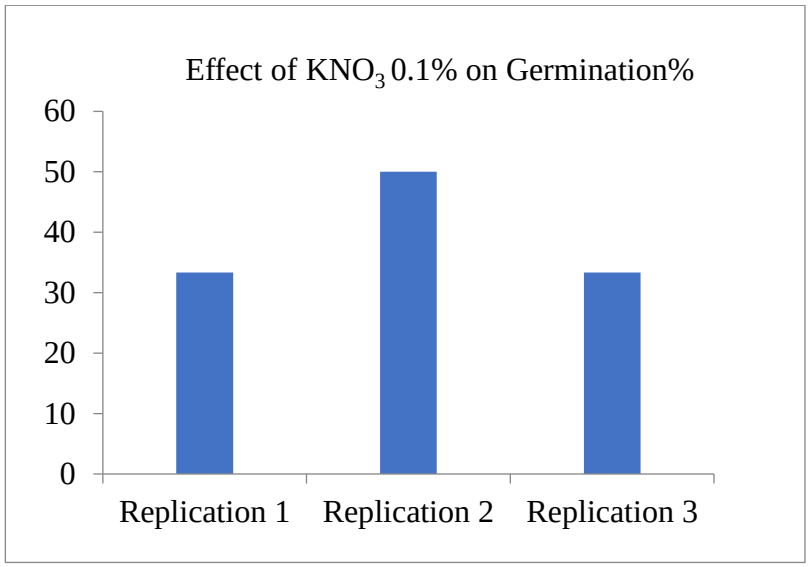
Effect of Potassium Nitrate on Seed Germination

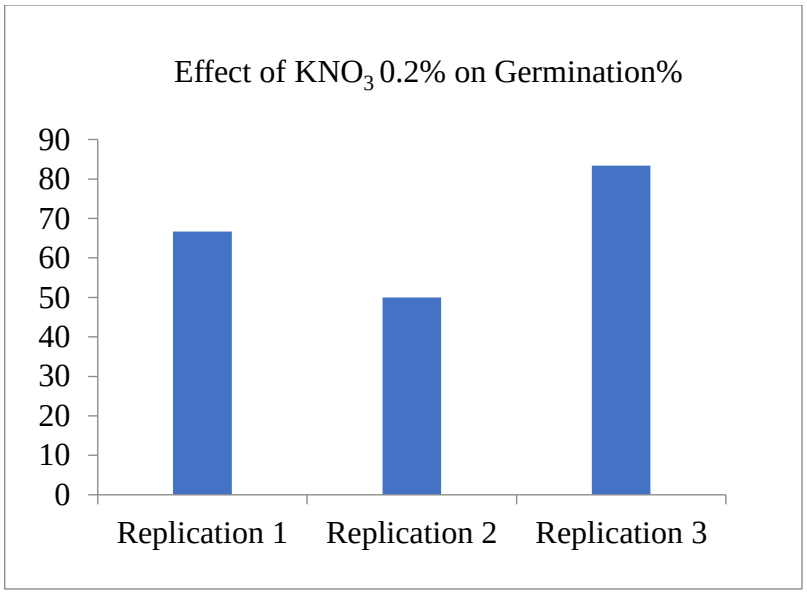
The use of potassium nitrate is used to improve seed germination. Figure 4.2 depicts the application of potassium nitrate. As shown in the figure the concentration of

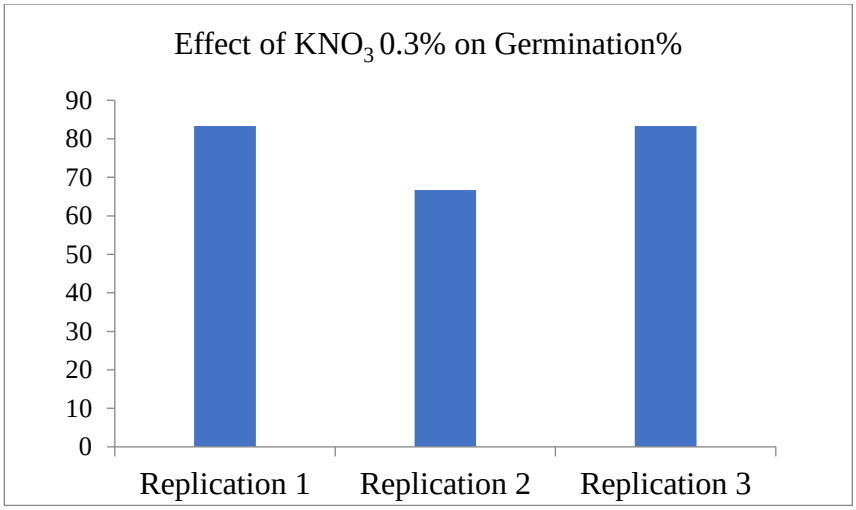
0.3% has a significant effect on the germination of *T. cypria* seeds. It enhances the germination rate of *T. cypria* seed to 77%. This concentration of potassium nitrate is significant among other concentrations of potassium nitrate as we increase the concentration of KNO_3. The germination rate of *T. cypria* seed increases.

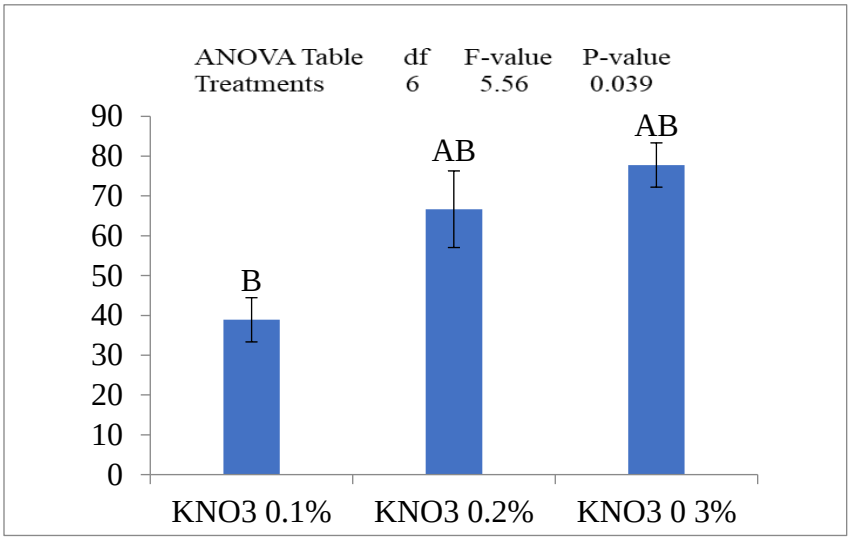
Figure 4.2

The Germination% with KNO_3 Potassium Nitrate effect.







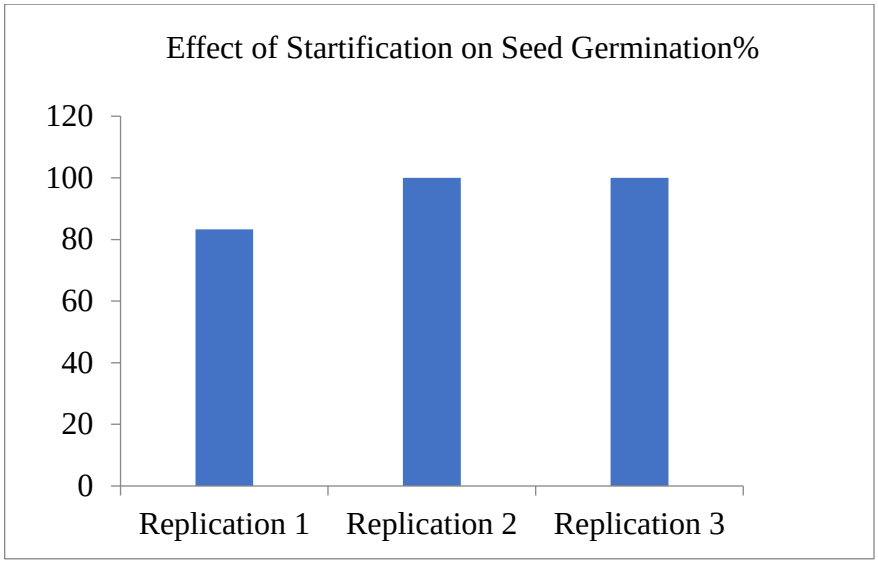


Effect of Cold Stratification on Seed Germination

The obtained result showed that Pre-chilling (stratification) increased seed germination rate in *T. cypria*. At 4°C temperatures, *T. cypria* seeds appear to give the highest percentage of 94% in seed germination ($P< 0.05$). As shown in Fig. 4.3 stratification had a greater germination rate than the GA3 and KNO3 treatments. The increase in temperature during stratification decreased the seed germination of Tulip and the highest germination percentage was observed at a constant temperature of 4°C. Data collected show that Pre-chilling improves seed FGP.

Figure 4.3

The effect of Stratification on Germination (%) of T. Cypria seeds



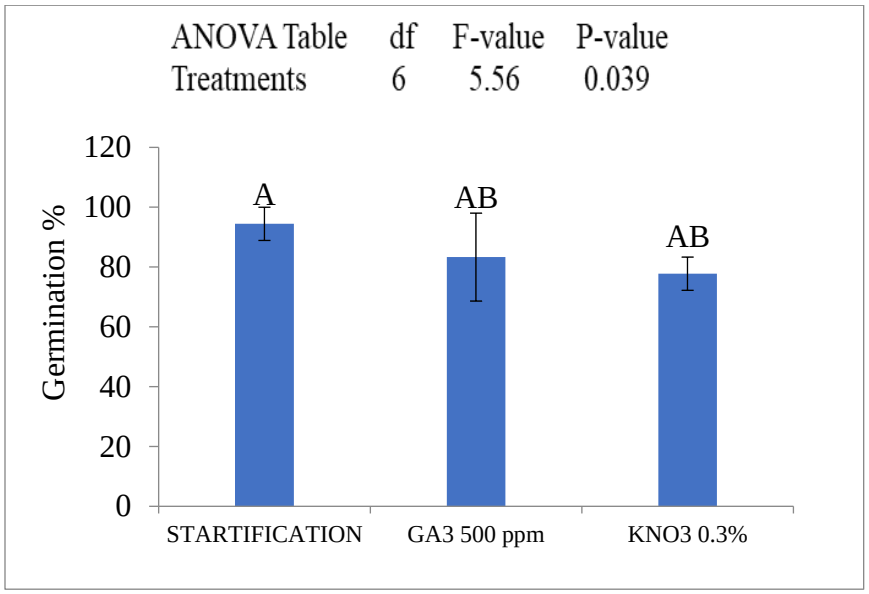


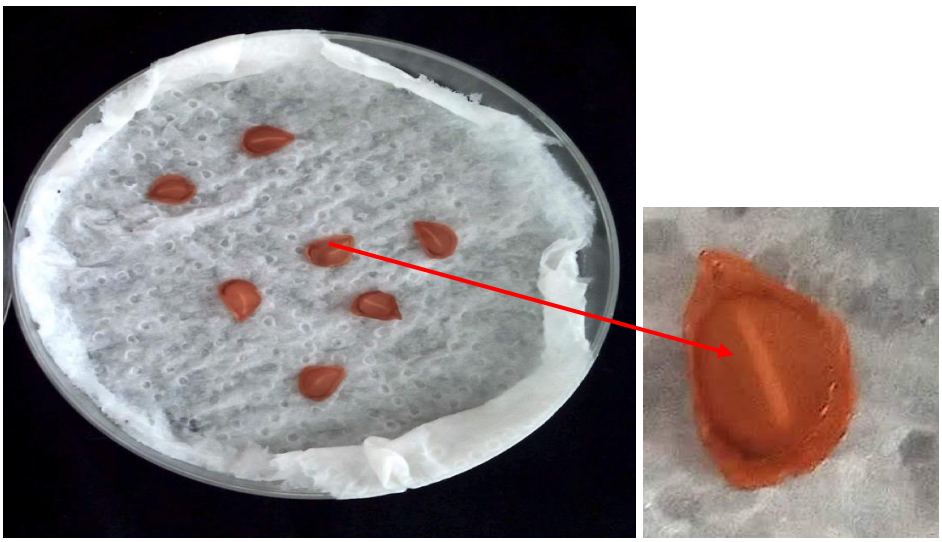
Table 4.1

Effect of seed dormancy breaking treatment on germination % of T. cypria

Dormancy breaking treatments	Final germination percentage%
Stratification	94.4444[a]
Gibberellic acid (GA_3)	
100ppm	61.1111[ab]
300ppm	44.44444[b]
500ppm	83.33333[ab]
Potassium nitrate (KNO_3 v/v)	
0.1%	38.88889[b]
0.2%	66.66667[ab]
0.3%	77.77778[ab]

Figure 4.4

Germination in T. cypria Seed. Germinated seeds and Embryo Germination (A, B) and germinated radicle (C).



(A) (B)

(C)

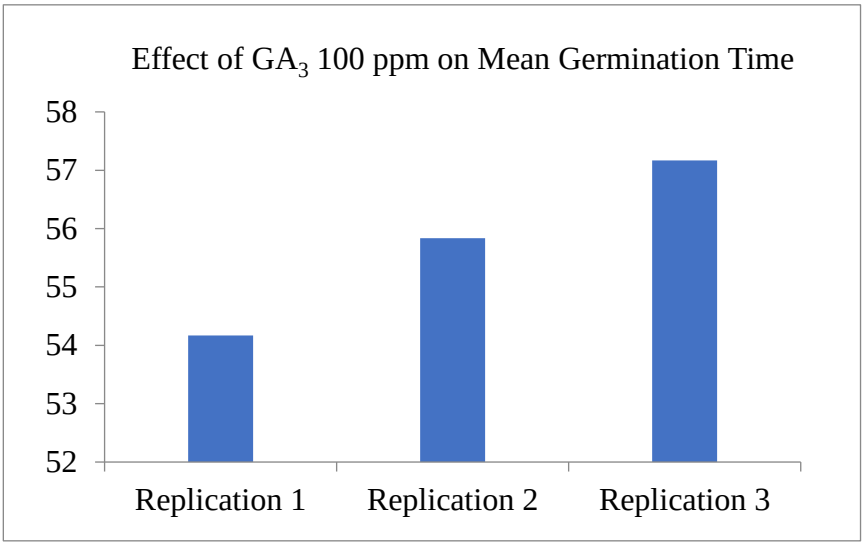
Effect of Gibberellic acid on Mean Germination Time

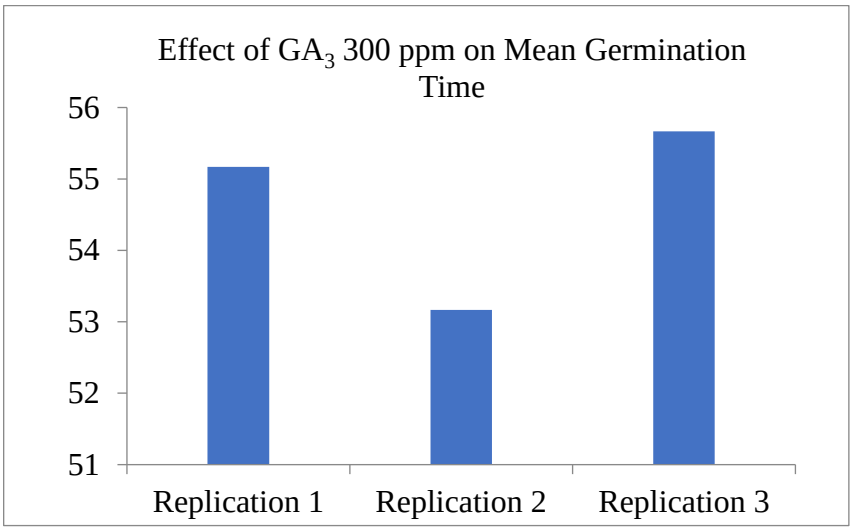
Gibberellic acid has a significant effect on the MGT of *T.cypria* seeds. Figure 4.5 depicts the influence of different concentrations of Gibberellic acid on seed MGT. Seeds primed with 500 ppm of solution germinated significantly faster than seeds primed with 100 ppm of solution. There is a significant difference between the concentrations of 100 ppm, 300, and 500 ppm on the MGT of *T. cypria* seeds. As you can see in Figure 4.5 the

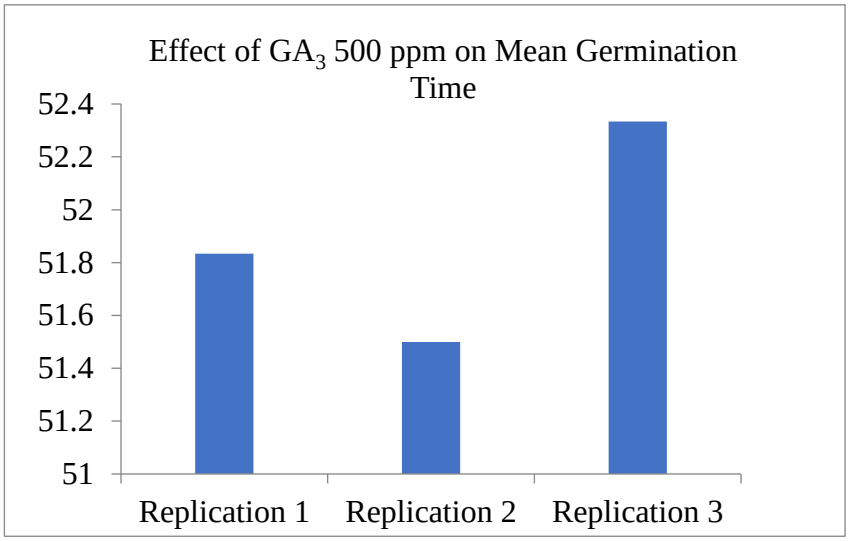
100ppm concentration affected the seed germination. Different germination times were observed in one concentration of Gibberellic acid solution.

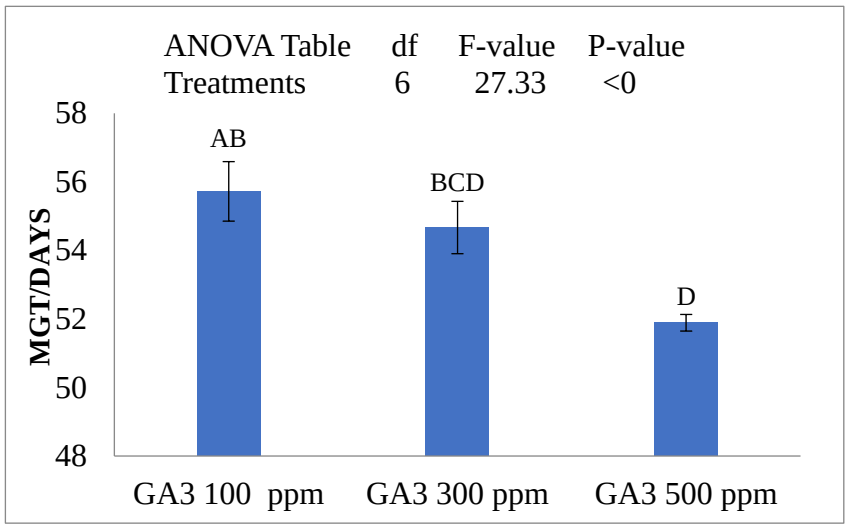
Figure 4.5

The Mean Germination time with GA_3 (gibberellic acid) effect.







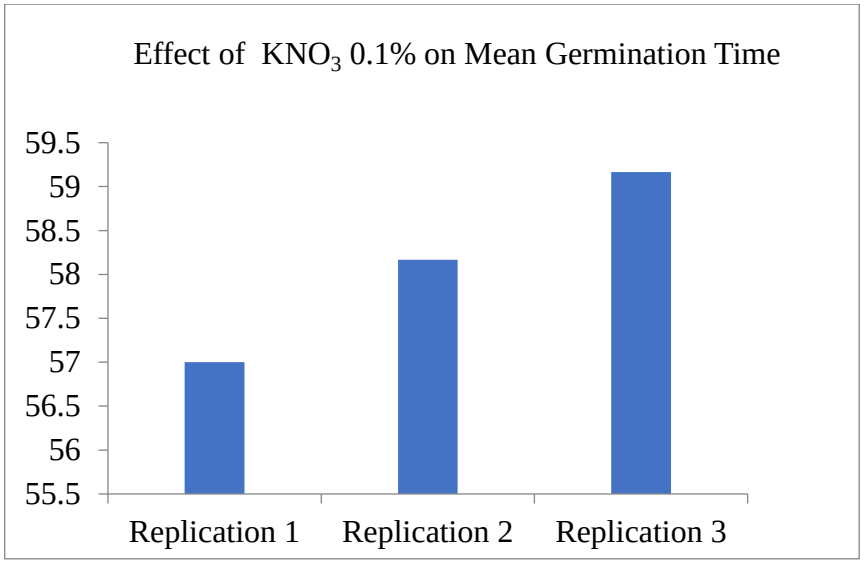


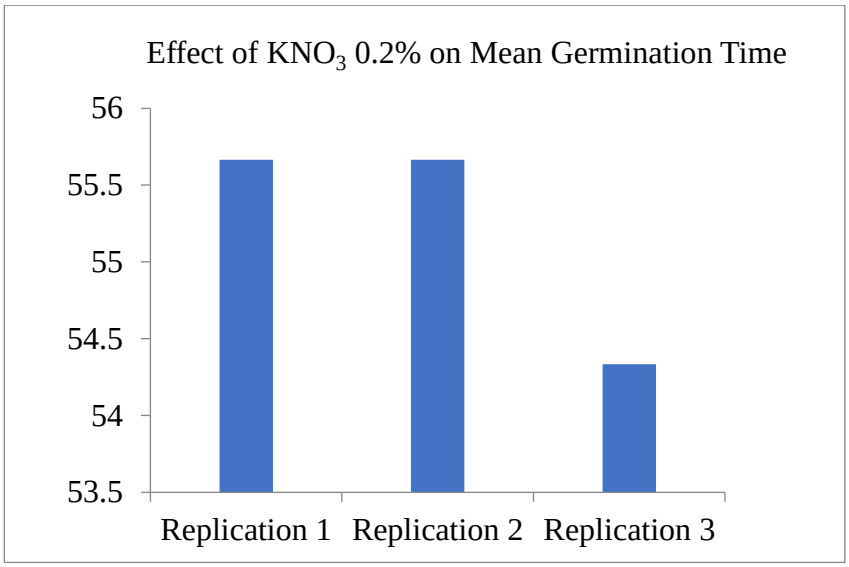
Effect of Potassium Nitrate on Mean Germination Time

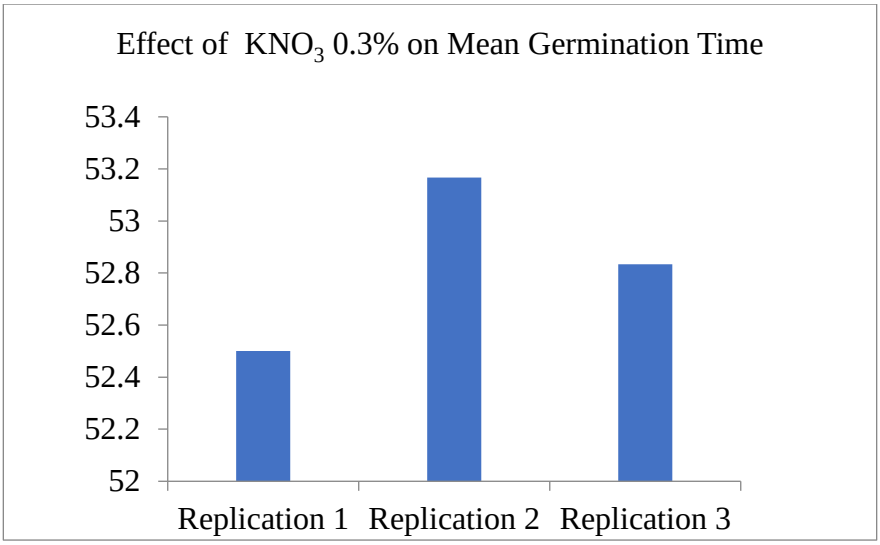
The MGT of *T. cypria* seeds is likewise influenced by treating the seeds with 0.1%, 0.2%, and 0.5% potassium nitrate solutions. Figure 4.6 depicts the influence of potassium nitrate on seed MGT. There is no significant change in MGT between seeds with concentrations of 0.1% 0.2% and 0.3%, as indicated in Fig 4.6 Seeds treated with 0.3% potassium nitrate, on the other hand, had a considerable effect on seed MGT. Furthermore, there is a negative significant relationship between MGT and FGP in *T. cypria* seeds. This indicates that as the germination % increased, so did the germination time decrease.

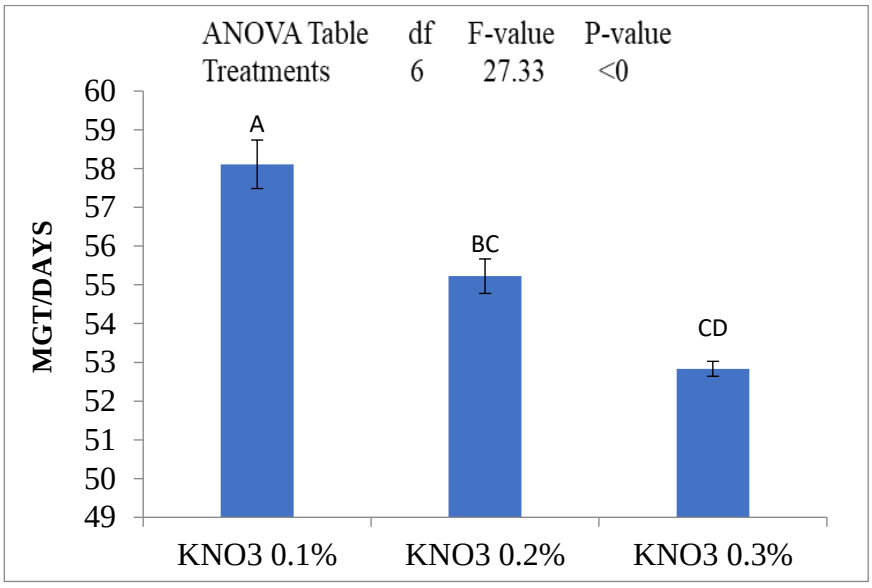
Figure 4.6

The Mean Germination time with KNO_3 (Potassium nitrate) effect









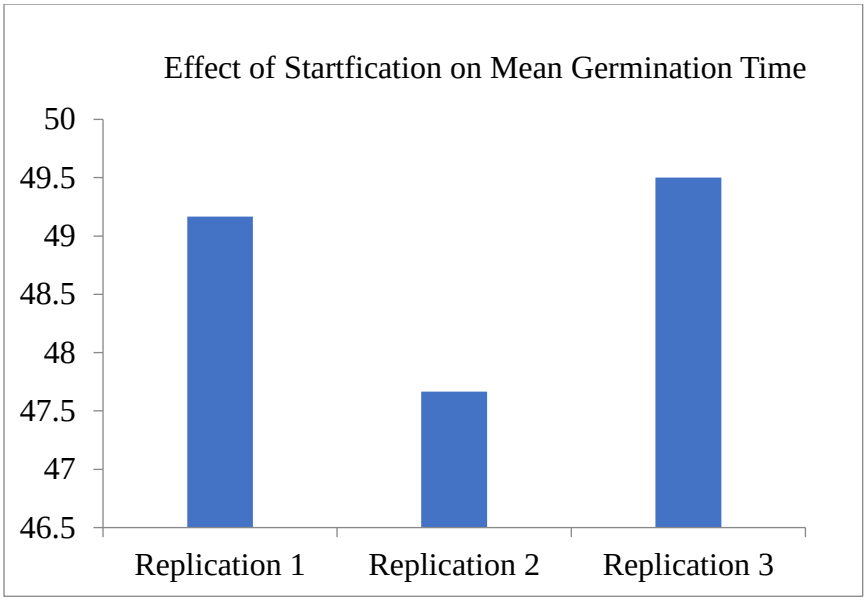
Effect of Stratification on Mean Germination Time

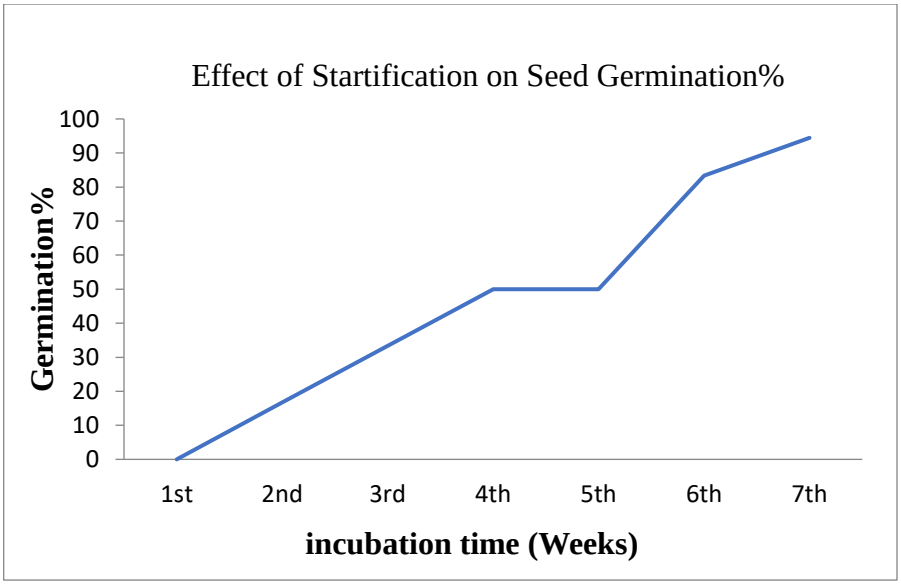
The germination time of *T. cypria* seed is significantly affected by the stratification procedure. In comparison to the other GA_3 and KNO_3 treatments, the *T. cypria* seeds germinated in 7 weeks. The MGT of stratification seed differs little from that of GA_3 priming seed. The MGT of stratification and seed primed with 0.3% KNO_3 are notably different. There was no seed germination recorded in the first week. There is 50% of germination observed after 25 days of incubation. As shown in the graph the germination gradually increases with time and attains a maximum germination within 6-7 weeks of incubation. it has been discovered that pre-chilling or cold temperatures have a significant impact on seeds.

The MGT of *T. cypria* seeds is likewise influenced by treating the seeds with 0.1%, 0.3%, and 0.5% potassium nitrate solutions. Figure 4.6 depicts the influence of potassium nitrate on seed MGT. There is no significant change in MGT between seeds with concentrations of 0.1% and 0.3%, as indicated in Fig 4.6. Seeds treated with 0.5% potassium nitrate, on the other hand, had a considerable effect on seed MGT. Furthermore, there is a negative significant relationship between MGT and FGP in *T. cypria* seeds. This indicates that as the germination % increased, so did the germination time decrease.

Figure 4.7

The Mean Germination time with KNO_3 (Potassium nitrate) effect




Effect of Startification on Seed Germination%
Germination%
100
90
80
70
60
50
40
30
20
10
0
1st
2nd
3rd
4th
5th
6th
7th
incubation time (Weeks)

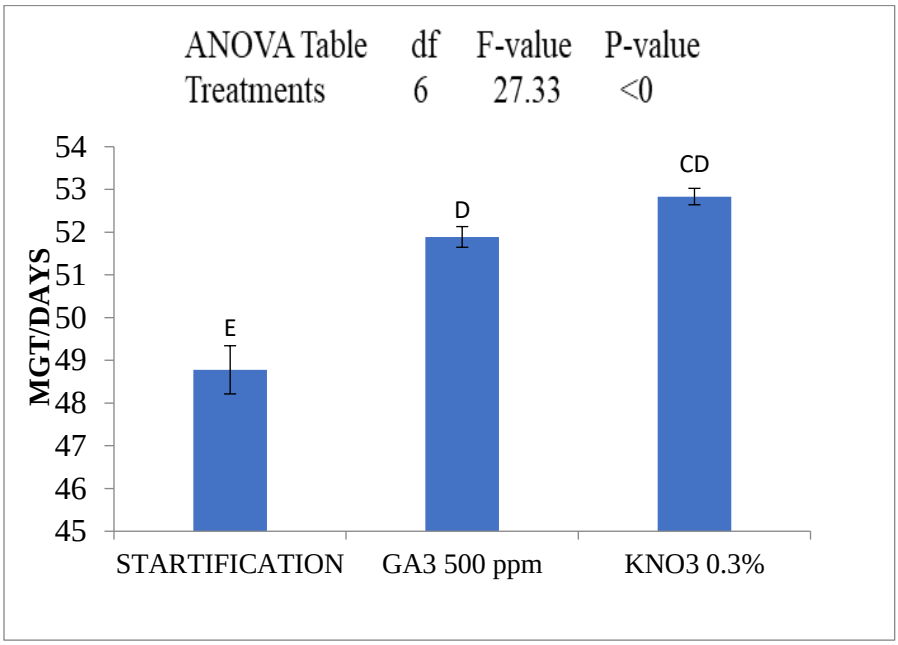

ANOVA Table df F-value P-value
Treatments 6 27.33 <0
MGT/DAYS
54
53
52
51
50
49
48
47
46
45
E
D
CD
STARTIFICATION
GA3 500 ppm
KNO3 0.3%

Table 4.2

Effect of seed dormancy breaking treatment on Mean germination time (Days) of *T. Cypria*

Dormancy breaking treatments	Mean germination time (Days)
Stratification	49[e]
Gibberellic acid (GA_3)	
100ppm	56[ab]
300ppm	55[bcd]
500ppm	52[d]
Potassium nitrate (KNO_3 v/v)	
0.1%	58[a]
0.2%	55[b]
0.3%	52[cd]

Discussions

Cold stratification has proven to be an effective approach for lowering seed dormancy (Zhang et al., 2020). It significantly improved *T. Cypria* seed germination in our testing. Rouhi et al. (2010) similarly concluded that 49-day stratification was effective in *T. Kaufmanniana* Regel. Our investigation found that the seed had a maximum germination rate of 96% at 4°C and required 25 days for partial germination. After 49 days of cold stratification, almost all of the seeds germinated. Both *T. hageri and T. orphanidea* seeds germinated at 5°C, indicating that a temperature of 4-5°C results in the highest proportion of germination (Pipinis et al., 2023). *T. Cypria* germinates optimally at a steady temperature of 4°C. These results show that *T. Cypria* seeds survived in the soil for a long time over the hot summer and germinated in early spring when the temperatures were favorable. Our findings also show that the time of stratification at constant temperature affects seed germination rates.

Phytohormones, such as gibberellin (GA) and abscisic acid (ABA), have a role in stimulating seed germination. It has been confirmed that the combination had a significant effect on seed germination (Shu, Liu, Xie, and He, 2016). Gibberellin helps to disrupt seed dormancy by rupturing the seed's testa (Lieu et al., 2016). GA_3 promotes dormant seeds in a variety of plant species by inducing hydrolytic enzymes that break down dormancy (Leubner-Metzger, 2001). GA_3 promotes seed germination by increasing protein synthesis and other metabolites in the embryo (Kupera et al., 2005). Our findings showed that 500 ppm GA_3 greatly improved the germination percentage and mean germination time of *T. cypria*. Furthermore, it differed from previous concentrations of 300 and 100 ppm GA_3. The other concentration produced less germination and took longer to germinate. The current study also found that applying KNO3 increased seed germination while decreasing the mean germination time. The KNO_3 impacts seed germination by regulating seed hormones, which leads to a reduction in germination inhibitors such as abscisic acid (Gashi et al., 2012). According to Tang et al. (2009) nitrogenous compounds can break seed dormancy by lowering the C6 Cl1 ratio of CO and changing the Because they accelerate the metabolic process, they are often used as germination promoters. It is thought that potassium nitrate can enter the embryo and boost metabolic activity. Rouhi et al. (2010) discovered that nitrogenous compounds have a regulatory role in breaking seed dormancy in various plant species. Our findings also revealed that potassium nitrate affects seed germination and mean germination time. As shown in Fig 4.6 the concentration of KNO_3 with 0.3% of concentration took less time to germinate as compared to other concentrations of 0.1% and 0.2%.

CHAPTER V

Conclusion and Recommendations

T. cypria seeds are dormant, and using pre-chilling and plant growth regulators such as Giberallic Acid and potassium nitrate influences the breaking of *T. cypria* seed dormancy. The germination rate increased by 94% during the stratification procedure compared to the plant growth regulator technique. The treatment of GA_3 and potassium nitrate considerably influences germination, but the stratification procedure works significantly better. The stratification process also enhances the rate of germination (MGT). As opposed to the two treatments of GA_3 and KNO_3, this took 7 weeks to germinate.

The outcomes of this study could serve as a beginning point for propagating these species for cultivation, commercial, or ornamental use. On the other hand, the multiplication of these species could significantly contribute to their conservation or direct preservation by reducing the harm caused by wild collection and preserving ex-situ stocks of these threatened species safely and cost-effectively. *Tulipa cypria* conservation includes a variety of actions to maintain and preserve this endangered plant species. Typical efforts include habitat restoration, managed agriculture, and wild population monitoring. Collaborative efforts among government agencies, conservation organizations, and local communities are critical to saving the Cyprus tulip from further decline. Public awareness initiatives highlight the need to maintain Cyprus's unique biodiversity.

Recommendations for Future Studies

For future studies researchers can work on the following fields related to study:

- ✓ Different seed breaking dormancy techniques can be used to enhance reproduction and effects on germination.
- ✓ Bulbs can be produced from seeds and can use different growth regulators to enhance the production through bulbs.

REFERENCES

Alboresi, A., Gestin, C., Leydecker, M.-T., Bedu, M., Meyer, C., & Truong, H.-N. (2005). Nitrate, a signal relieving seed dormancy in Arabidopsis. *Plant, cell & environment*, 500-512.

Bethke, P. C., Libourel, I. G., & Jones, R. L. (2007). Nitric oxide in seed dormancy and germination. *Annual Plant Reviews Volume 27: Seed Development, Dormancy and Germination*, 153-175.

Bewley, D. J. (1997). *Seed germination and dormancy.* Oxford University Press.

Bewley, D. J., & Black, M. (1994). *Seeds: germination, structure, and composition.* Springer.

Bilz, M. (2013). *Tulipa cypria. The IUCN Red List of Threatened Species 2013*. Retrieved from https://www.iucnredlist.org/species/161927/5514790

Buijs, G. (2020). A perspective on secondary seed dormancy in Arabidopsis thaliana. *Plants*, 749.

Christenhusz, M. J., GOVAERTS, R., DAVID, J. C., HALL, T., BORLAND, K., ROBERTS, P. S., . . . FAY, M. F. (2013). Tiptoe through the tulips--cultural history, molecular phylogenetics and classification of Tulipa (Liliaceae). 280-328.

Donato, L., Stefano, C., & Adérito Tomás, P. (2019). Germination of Chloris barbata, Cynodon dactylon, and Cyperus rotundus from Angola at constant and alternate temperatures. *Agronomy*, 615.

Finch-Savage, W., & Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New phytologist*, 501-523.

Finkelstein, R., Reeves, W., Ariizumi, T., & Steber, C. (2008). Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.*, 387-415.

GASHI, B., OSMANI, M., & Sali, A. (2019). Breaking seed dormancy of Tulipa scardica Bornm. and Tulipa kosovarica Kit Tan, Shuka \& Krasniqi by

pre-chilling, plant growth regulators and some chemical treatments. *Acta agriculturae Slovenica*, 203-210.

Geneve, R. L. (1998). Seed dormancy in commercial vegetable and flower species. *Seed Technology*, 236-250.

Govaerts, R., Paton, A., Harvey, Y., & Navarro, T. (2017). World checklist of Lamiaceae. Royal Botanic Gardens, Kew.

Govaerts, R., Paton, A., Harvey, Y., & Navarro, T. (2017). World checklist of Lamiaceae. Royal Botanic Gardens, Kew.

Hermann, K., Meinhard, J., Dobrev, P., Linkies, A., Pesek, B., Heß, B., . . . Leubner-Metzger, G. (2007). 1-Aminocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (Beta vulgaris L.): a comparative study of fruits and seeds. *Journal of Experimental Botany*, 3047-3060.

HR Rouhi, F. K. (2012). Effects of sulfuric acid, stratification, phytohormone and potassium nitrate on dormancy breaking and germination of water lily tulip (Tulipa kaufmanniana Regel. *International Journal of AgriScience*, 136-142.

Joshi, M., & Dhar, U. (2023). Effect of various presowing treatments on seed germination of Heracleum candicans Wall. ex DC.: a high value medicinal plant. *Seed Science and Technology*, 112-116.

Loddo, D., Carlesi, S., & Pais da Cunha, A. (2019). Germination of Chloris barbata, Cynodon dactylon, and Cyperus rotundus from Angola at constant and alternate temperatures. *Agronomy*, 615.

Meikle, R. (1985). Flora of Cyprus, the Bentham-Moxton Trust. *Royal Botanic Garden, Kew (2 vol.)*.

Miransari, M., & Smith, D. (2014). Plant hormones and seed germination. *Environmental and experimental botany*, 110-121.

Nkomo, M. M., & Kambizi, L. (2009). Effects of pre-chilling and temperature on seed germination of Corchorus olitorius L.(Tiliaceae)(Jew's Mallow), a wild leafy vegetable. *African Journal of Biotechnology*.

Orlikowska, T. a.-C. (2018). Tulip. *Ornamental crops*, 769-802.

Pipinis, E., Hatzilazarou, S., Kostas, S., Stagiopoulou, R., Gitsa, K., Dariotis, E., . . . Bareka, P. (2023). Effect of Temperature on Breaking of Morphophysiological Dormancy and Seed Germination Leading to Bulblet Production in Two Endemic Tulip Species from Greece. *Plants*.

Rhie, Y., & Lee, S. (2020). Seed dormancy and germination of Epimedium koreanum Nakai. *Scientia Horticulturae*.

Rouhi, H., Karimi, F., Shahbodaghlo, A., Sheikhalian, M., Rahmatabadi, R., Samadi, M., & Karimi, F. (2012). Effects of sulfuric acid, stratification, phytohormone and potassium nitrate on dormancy breaking and germination of water lily tulip Tulipa kaufmanniana Regel. *International Journal of AgriScience*, 136-142.

Rouhi, H., Shakarami, K., & Afshari, T. R. (2010). Seed Treatments to Overcome Dormancy of Waterlily Tulip ('Tulipa kaufmanniana'Regel). *Australian Journal of Crop Science*, 718-721.

Shanmugavalli, M., Renganayaki, P., & Menaka, C. (2007). Seed dormancy and germination improvement treatments in fodder sorghum.

Sixtus, C., Hill, G., & Scott, R. (2003). The effect of temperature and scarification method on gorse (Ulex europaeus L) seed germination. *New Zealand plant protection*, 201-205.

Stefanaki, A. W. (2022). Tracing the introduction history of the tulip that went wild (Tulipa sylvestris) in sixteenth-century Europe. *Scientific Reports*, 9786.

Tang, A.-j., Tian, M.-h., & Long, C.-l. (2009). Seed dormancy and germination of three herbaceous perennial desert ephemerals from the Junggar Basin, China. *Seed Science Research*, 183-189.

Thomas, S. G., Rieu, I., & Steber, C. M. (2005). Gibberellin metabolism and signaling. *Vitamins \& hormones*, 289-338.

TRIAS-BLASI, A., GÜCEL, S., & ÖZDEN, Ö. (2017). Current distribution and conservation status reassessment of the Cyprus Tulip (Tulipa cypria: Liliaceae), new data from Northern Cyprus. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*.

Tsintides, T., Christodoulou, C., & Delipetrou Georghiou, K. (2007). *The red data book of the flora of Cyprus Cyprus Forest Association.*

Van Raamsdonk, L., & De Vries, T. (1995). Species relationships and taxonomy in Tulipa subg. Tulipa (Liliaceae). *Plant Systematics and Evolution*, 13-44.

Wilford, R. (2006). *Tulips: species and hybrids for the gardener*. Timber Press (OR).

Xing, G., Qu, L., Zhang, Y., Xue, L., Su, J., & Lei, J. (2017). Collection and evaluation of wild tulip (Tulipa spp.) resources in China. *Genetic resources and crop evolution*.

Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y., & Yamaguchi, S. (2004). Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of Arabidopsis thaliana seeds. *The Plant Cell*, 367-378.

Zhang, W., Qu, L.-W., Zhao, J., Xue, L., Dai, H.-P., Xing, G.-M., & Lei, J.-J. (2020). Practical methods for breaking seed dormancy in a wild ornamental tulip species Tulipa thianschanica Regel. *Agronomy*, 17-65.

APPENDIX

Similarity report