# GENETIC VARIATION PATTERN AMONG *ABIES CILICICA* (PINACEAE) POPULATIONS: ADAPTIVE SEED AND SEEDLING TRAITS

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### ABSTRACT

## GENETIC VARIATION PATTERN AMONG ABIES CILICICA (PINACEAE) POPULATIONS: ADAPTIVE SEED AND SEEDLING TRAITS

Gülsoy, Ali Murat Doctor of Philosophy, Biology Supervisor: Prof. Dr. Musa Doğan

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The pattern of genetic variation in adaptive seed and seedling traits of *Abies cilicica* was investigated using 160 families from 8 populations representing the species' natural range for two years in the Olcay Forest Nursery in Konya. The study indicated that both populations and families within populations varied significantly in seed and seedling traits. The pattern of genetic variation among populations suggests that there may be a clinal variation concerning altitude and latitude regarding these traits. However, further investigations need to be done beyond the two growing seasons. The variation due to populations varied from 0.3% in Diam20 to 41.5 % in Ht20, while the variance component of families within populations was from 12.4 % in BS20 to 85% in survival in the 2020 growing season.

Estimated family heritabilities were generally high for most traits, ranging from 0.41 in BS20 to 0.91 in BB20 and 0.98 in survival in 2020. Seed weight significantly and positively correlated with growth traits measured in 2019 and 2020, indicating the

presence of maternal effects on early performances of seedlings' growth. Genetic and phenotypic correlations between adaptive seedling traits in 2020 were in the same magnitude and direction. There were strong and negative genetic correlations between bud set date and growth traits in the 2020 growing season. Interestingly, there was strong and negative genetic correlation between BB20 and BS20 in the same growing season, meaning that those families with late bud bursts had early bud sets, having a short growing season. The amount and pattern of genetic diversity in seed and seedling traits and genetic correlations among them suggest that Cilician fir will have a great potential to adapt to changing climate and to provide opportunities for foresters to use effectively assisted migration practices.

Keywords: *Abies cilicica*, Nursery experiment, Genetic variation, Seed and seedling traits, Assisted migration

## *ABIES CILICICA* (PINACEAE) POPÜLASYONLARINDA GENETİK ÇEŞİTLİLİLİK YAPILANMASI: ADAPTİF TOHUM VE FİDAN KARAKTERİSTİKLERİ

ÖΖ

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*Abies cilicica*'nın adaptif tohum ve fidan özelliklerindeki genetik varyasyon biçimi, türün doğal yayılımını temsil eden 8 populasyondan 160 familya kullanılarak Olcay Orman Fidanlığı'nda iki yılda yürütülen bir fidanlık denemesinde araştırılmıştır. Çalışma, hem popülasyonların hem de popülasyonlardaki ailelerin tohum ve fidan açısından önemli ölçüde farklı olduğunu göstermiştir. Tohum ve fidan özelliklerinde, popülasyonlar arasındaki genetik varyasyon biçimi, yükseklik ve enlem açısından bir klinal varyasyon olabileceğini düşündürmektedir, ancak iki büyüme mevsiminin ötesinde, daha uzun soluklu araştırma yapılması gerekmektedir. Popülasyonlara bağlı varyasyon Diam20'de %0,3'ten Ht20'de %41,5'e kadar değişirken, popülasyonlar içindeki ailelere bağlı varyans bileşeni 2020 büyüme sezonunda BS20'de %12,2'den yaşama yüzdesinde %85'e kadar çıkmıştır. Tahmini aile kalıtsallıkları, BS20'de 0,40'a, BB20'de 0.98'e ve 2020'deki yaşama yüzdesinde 0,98'e kadar değişen aralıkta çoğu özellik için genellikle yüksek hesaplanmıştır. Tohum ağırlıklarının, fidanların erken büyüme performansları üzerinde tohum ağırlıklarının maternal etkilerinin varlığını gösteren, 2019 ve 2020'de ölçülen büyüme özellikleri ile anlamlı ve pozitif bir ilişkide olduğu bulunmuştur. 2020 yılındaki adaptif fidan özellikleri arasındaki genetik ve fenotipik korelasyonlar aynı büyüklük ve yönde olmuştur. 2020 büyüme sezonunda tomurcuklanma tarihi ile büyüme özellikleri arasında güçlü ve negatif genetik korelasyonlar görülmüştür. İlginç bir şekilde, aynı büyüme mevsiminde BB20 ve BS20 arasında güçlü ve negatif genetik korelasyon saptanmıştır. Bu, geç tomurcuk patlamaları olan ailelerin erken tomurcuk oluşturduklarına ve kısa büyüme sezonuna sahip olduklarını göstermektedir. Tohum ve fidan özelliklerindeki genetik çeşitliliğin büyüklüğü, biçimi ve aralarındaki genetik korelasyonlar, Kilikya göknarının değişen iklime uyum sağlamak için büyük bir potansiyele sahip olduğunu ve ormancıların bu türde destekli göç uygulamalarını etkin bir şekilde kullanabilmeleri için fırsatlar olduğunu ortaya koymaktadır.

Anahtar Kelimeler: *Abies cilicica*, Fidanlık denemesi, Genetik varyasyon, Tohum ve fidan özellikleri, Destekli göç

To my son, my wife, my mother, my father, and my aunt,

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## LIST OF ABBREVIATIONS

EUFORGEN: European Forest Genetic Resources Program

- IPCC: Intergovernmental Panel on Climate Change
- **BP:** Before Present
- G×E: Genotype x Environment
- ClimateEU: Climate Data for Europe
- ClimateWNA: Current and projected climate data for western North America
- ISTO: Herbarium of Faculty of Forestry, University of İstanbul
- GDF: General Directorate of Forestry in Turkey
- GCM: Global Circulation Model
- IUCN: International Union for Conservation of Nature
- SAS: Statistical Analysis System
- SMS: State Meteorological Service

# LIST OF SYMBOLS

# SYMBOLS

# P: Probability

# Ka: 1000 years

# HE: Heterozysity

## F<sub>ST</sub>: Fixation Index

# Mg: milligram

### **CHAPTER 1**

### **INTRODUCTION**

## 1.1. Cilician Fir

There are 48-49 species of firs (*Abies* Mill.) in the Coniferae class of the Pinaceae family in the world (Farjon, 2010). The Northern hemisphere's temperate and cool zones, including those in Europe, Asia, North Africa, and North and Central America, are home to firs. (Schopmeyer, 1989; Farjon, 2010). Turkey's natural species fall under the "*Abies* section" of the genus, which has eight sections (Farjon, 2010). Coode And Cullen (1965) divided Turkish firs into Taurus and Eastern Black Sea firs according to their shoots and buds and classified Uludağ and Kazdağı firs as subspecies of Eastern Black Sea fir. In high mountainous areas in southern latitudes, lower elevations, and even at sea level in northern latitudes, *Abies* taxa establish pure or mixed forests (Yaltırık and Efe 2000). Although Turkey's forests are poor in productivity, the firs are pretty rich in the number of species. In addition, an essential part of Turkey's natural species that make up forest lands is endemic species. Besides the importance, they enrich Turkey's flora. Endemic species also carry individual responsibility for Turkey's forestry practices.

One of the fir species that naturally grows in Turkey is the Cilician or Taurus fir (*Abies cilicica* Carr.) (Kayacık, 1967; Yaltırık, 1993). History, culture, the arts, science, and the economy all recognize its importance. (Bozkuş, 1988). It is an indigenous conifer tree species that lives in high mountain environments in southern Turkey, northern Lebanon, and the northwest of Syria. (Awad et al. 2014) (Figure 1.1). The scientific categorization of the species is exceptionally complicated due to the extraordinary inconsistancy of morphological characteristics. (Bozkuş and Çoban,

2006; Yaltırık and Akkemik, 2011). Additionally, there are two subspecies of *A. ci-licica* (Ant. & Kotschy). Young branches and resinless buds are characteristics of *Abies cilicica* Carr subsp. *cilicica*. However, the buds and young branches of *A. ci-licica* (Ant. & Kotschy) Carr. subsp. *isaurica* Coode & Cullen are resinous. This second subspecies (subsp. *isaurica*) is endemic to the Eastern Mediterranean (Davis et al., 1965).



*Figure 1.1.* Distribution map of Cilician fir (EUFORGEN 2021;<u>http://www.euforgen.org/species/abies-cilicia/</u>) Retrieved on 26.7.2022

Cilician fir, a critical distribution area in Turkey, is valuable in landscaping with its decorative appearance in parks and gardens. It is preferred like other fir species because it has the desired properties in use as a Christmas tree (Atay, 1988). In addition, studies on wood morphology and technology of this species and paper technology with its fiber properties show that it is an important raw material, especially in the

packaging industry and construction sector, due to its lightweight (Aytuğ, 1959; Tank, 1964; Bozkurt, 1971).

#### 1.2. Biology, natural range, and economic importance

If the taproot does not rot, the Cilician fir, a species of primary forest tree, can live for 250–300 years and reach heights of 40–42 meters with a diameter of 1.5 meters. In advanced ages, the crown is narrow and steep. The branches form acute angles to the trunk at the tops of the crown. In the lower half, they come out with a wide angle (Bozkuş, 1988). They are evergreen forest trees whose branches are regular until old age (Kayacık, 1980; Yaltırık, 1988). The ash-gray bark, which is smooth in youth, later cracks and flakes (Yaltırık, 1988) and becomes deeply fissured with age (Kayacık, 1980). The leaves are linear-oblong, notched, blunt, or pointed. The leaf color is usually light green (Kayacık, 1980; Yaltırık, 1988). The needle-like leaves are straight and aligned on the margins at the top of the shoots, 2-4 cm long, bright green upper side; the tip is pointed, blunt or notched. In the leaves, a resin channel is just under the epidermis, sometimes a little deeper, close to the edge. Usually, no stomata are present on the upper part of the leaves; on the lower face, there are greenish-white stomatal bands consisting of six or seven. Male flower cones, found where the leaves on the shoot are located, are 10-15 mm long, consisting of small assemblages and red (Eckenwalder, 2009). Male flower panels are primarily located in the lower and middle parts of the crown. The female flower panels are in small cones at the top of the peak (Akkemik, 2012). The upright, resin-rich cones are 15–25 cm in length and 4-6 cm in diameter. Cilician fir possess the largest cone among the natural fir taxa of Turkey. Unlike Abies *nordmanniana*, since the outer scale is shorter than the inner scale, the outer scale is not visible from the outside when the cone is closed (Coode and Cullen, 1965; Kayacık, 1980; Yaltırık, 1988). Young shoots are gray-brown, shiny, bare, or with fine brown hairs. Depending on the growing environmental conditions, the shoots are thicker in slow-growing individuals. The apical bud (terminal bud) and the subterminal buds closest to the apex are resinous or irregular (Coode and Cullen, 1965; Kayacık, 1980; Yaltırık, 1988). The leaf base is corrugated. Buds are 3-4 mm long, unregulated, or less

hairy (Eckenwalder, 2009, Farjon, 2010). The general view of the species and some of its features are shown in Figure 1.2.



*Figure 1.2.* A. *Abies cilicica*, B. Mature female cones, C. Male catkins, D. Needlelike leaves (Akkemik, 2012).

Cilician fir also thrives in humid climates and fertile soils, according to common fir tree features. The shade-resistant fir trees may endure prolonged, severe shade for 100 to 150 years before emerging to experience fast development. It creates tiny annual rings in years when it is in the shadow. The Taurus Mountains' northern and southern sides have Cilician fir, stretching from 1300 to 1400 meters above sea level. While the Cilician fir forms pure stands in the Mediterranean Region, it mixes with

Anatolian black pine, Taurus cedar, Syrian juniper (*Juniperus drupacea*), foetid juniper (*Juniperus foetidissima*), Greek juniper (*Juniperus excelsa*), and Turkish red pine and oaks at extreme limits of its natural distribution (Akkemik, 2012). This species' geographical range is rather small. (M'Hirit 1999).

Furniture, paneling, boxes, bundles, linens, and toys have all been made from the wood of *A. cilicica*.

### 1.3. Conservation and breeding works

Firs are tall forest trees that are pyramidal at a young age, then evergreen with a conical apex and branches arranged around the trunk. Fir forests are very wind and storm resistant because trees start producing tap roots at a young age. The soil and moisture requirements of firs are high; their heat demand is moderate, while they are sensitive to spring frosts. Their light conditions are low, and they are shade-tolerant. (Sarıbaş, 2011).

The environment, which was more humid in the Mediterranean basin than today, caused the disappearance of fir in the low-altitude regions due to the gradual aridification, but caused it to remain in small or large communities in suitable places. In other words, it is stated that today's disconnected, isolated distribution areas firs spread in Europe and Asia Minor were once shared. Firs might be present in intermediate regions where no fir species existed today when the climate was suitable (Mattfeld, 1928). Like all forest areas, Cilician fir stands are under heavy pressure from unplanned and purposeless interventions in the Mediterranean region, one of the oldest settlement areas in the world. After the period of forestry contractor management, it cannot be said that sufficient technical and silvicultural measures have been implemented to stop this trend. In the region, cases that have been purified or transformed into degraded fir stands by the reduction or complete destruction of basic stand elements such as Anatolian black pine and cedar, through interventions

aimed at allowable cut, are quite common. Also, there has been no significant intervention for the cultivation and development of pure and degraded fir stands in the region to date. At this stage, all Cilician fir stands are able to be said in the natural forest character (Bozkuş, 1988). These woodlands are less threatened than those in Lebanon and Syria. In any case, expansive zones of the woods are in a state of debasement. In addition, 86 percent of mixed stand forest areas and 44 percent of temperate, pure forest lands are both degraded (Bozkus 1988). Goat overgrazing has been a major contributor to this. Goats are especially dangerous since the areas in which they feed cannot effectively recover after grazing, which causes them to injure seedlings and young shoots (McGinley 2008). Other risks include pathogen attacks, which is partly brought on by increased tourism in the Taurus Mountains, and fire. Additionally, unexpected deaths of *A. cilicica* subsp. *isaurica* have been reported (Carus 2010), and increasing summer temperatures and decreased precipitation both have led to a common decline in woodland wellness that are linked to global warming. (Öztürk, 2010).

The Cilician fir is very selective regarding its vital requirements such as light, heat, moisture, soil structure, and texture. It needs a trench at the beginning of afforestation and the zoo-biotic factors that prevent seedlings' development. These requirements must be considered to have the continuity of this species. Although Cilician fir grows in a hotter and drier region compared to other fir species of Turkey, its best stands are located in the coolest and humid high parts of its natural range and in the northern aspects that are not exposed to the sun much. It is possible to explain the scattered and fragmented distribution of the Cilician fir by its regression to suitable parts due to the negative changes in climatic conditions throughout geological periods (Bozkuş, 1988).

Although the Turkish Constitution's articles do not expressly address the preservation of plant genetic resources with different qualities, article 63 of the Constitution, which Turkey ratified on October 18, 1982, states that the government must support and protect preservation efforts in addition to protecting its genuine, social, and shared values and assets. The Law on Protection of Cultural and Natural Assets (Code: 2863 - 23.07.1983), the Environment Law (Code: 2872, 1983), the National Parks Laws (Code: 2873, 1993), the Law of Special Environment Protection Regions (Code: 88/13019, 1988), and the Forestry Law (Codes: 6831) provide for the security of nature and various natural qualities and advance activities related to the preservation of inherited assets (Kaya and Raynold, 2001).

Moreover, changed government directions, such as The Control on the Collection, Capacity, and Utilize of Plant Genetic Resources (1992, The Turkish Official Gazette), limit the overexploitation of hereditary assets (Kaya et al., 1997).

To safeguard the Cilician fir in Turkey, three seed stands and seven gene conservation forests have been developed within the bounds of the aforementioned legal bases (Ministry of Agriculture and Forestry, 2021). Additionally, it is said that a workable plan must be established to preserve and maintain the genetic resources of the Cilician fir, which is also grown in Turkey. The stands of this species are managed because of the Cilician fir's high economic value in Turkey. In order to prevent overexploitation and further loss of genetic diversity, future efforts should focus on monitoring the demography and genetic threats to the existing populations. (Sekiewicz et al., 2015).

### 1.4. Climate change and adaptability of firs and specifically Cilician fir

Climate can be a vital factor shaping the woodland environment. Therefore, climate changes affect the physiology, development, mortality, and formation of trees, interactions between trees and pathogens, and ultimately disturbance management (winds, forest fires, insect attacks, etc.). The vulnerability of such changes depends on the level considered (woods versus landscapes, single trees versus stands) and specific site conditions (Bugmann et al., 2014). With the human impact on climate

becoming increasingly clear (IPCC, 2013), the results of modified temperature and precipitation administrations for trees and woodland biological systems are distinctively discussed. In Europe, the rise in mean yearly temperature as of now is more than 1.1 °C compared to the preindustrial normal, surpassing the cruel worldwide increment of 0.78 °C (IPCC 2007, 2013). The frequency of hot days has nearly tripled while the average length of warm summer waves over Western Europe has increased. (Della-Marta et al., 2007b). In timberlands, the dry season could be a significant constraint to plant development and efficiency because it is for most earthly plant communities (Churkina & Running, 1998; Luyssaert et al., 2010).

Modern investigation shows numerous plant species cannot develop good versatility to adjust to climate change. In this manner, most of them will be vulnerable to compressions in their ranges/geographical limits and a decrease in their populations (Naudiyal et al., 2021). Among species with local conveyances, it has been detailed that there will be significant habitat shrinkages and biodiversity misfortunes (Dinerstein et al., 2020; Wang et al., 2019). In expansion, a few consider that climate change will lead a few species to emigrate, especially towards the poles or high elevations, in which species' strength might increment over time. (Du et al., 2021; Rana et al., 2020; Yi et al., 2016). Plants are not likely to be able to create the physiological resistances vital to adjust to climate change quickly. Future range shifts must be considered, along with organic responses to natural change and dynamic forms like environment fracturing, as well as their effects on the average scene (Yesuf et al., 2021; Lindermayer et al., 2007).

With 25,000 plant species, more than half of which are endemic, the Eastern Mediterranean Basin is the largest area with a Mediterranean climate and one of the world's most significant climate (Medail & Quezel, 1997). The number of tall plants in the Mediterranean is significantly influenced by trees. For instance, 245 species and subspecies of trees have been reported in the Mediterranean, compared to 30 tree species in Europe's mild climate (Médail et al., 2019). Due to its exceptional biodiversity, this area is now considered to be one of the world's biodiversity hotspots, which is essential for large-scale programs of nature conservation (Mittenmeier et al., 2011; Albassatneh et al., 2021).

The reduction in precipitation has been identified in recent reports and estimates about global climate change as the most significant threat to the stability of the majority of recognizable biological systems, with important implications for human cultures. Almost all Mediterranean locations will experience various dry season occurrences of varying magnitude and intensity. In this regard, it has been acknowledged that the Mediterranean basin is one of the most vulnerable regions to future global warming. It has been labeled as a climate change hotspot (Hoerling et al., 2012; Spinoni et al., 2018). In the future, droughts are predicted to occur more frequently, linger longer, and be more severe. The precipitation slant within the Mediterranean basin from 1960 to 2005, which excludes the most recent decades, was 22 mm per decade (Mariotti et al. 2008). The plan is unstable because certain Mediterranean regions would see unfortunate early precipitation towards the end of the century, while others may get more rain (Zittis, 2018). Many indigenous plant species in the area, which currently have a restricted and fragmented range and occur in specific habitats, depend on maintaining this dry drift inside the Mediterranean. In this regard, tree species that are developing in the mountain belts of the Mediterranean, such as coniferous species, may have a high possibility of degenerating (Ruiz-Labourdette et al., 2013; Marqués et al., 2016; Navarro-Cerrillo et al. 2020).

Mediterranean firs are regularly found in ensured regions because of their endemism level and restricted dispersion or their crucial part as cornerstone species in Mediterranean mountain biological systems (Alizoti et al., 2011).

In the Mediterranean, endemism and biodiversity are not distributed equally, and a few local hotspots have been identified, including the East Mediterranean, which is the richest territorial hotspot (Medail & Quezel, 1997). With an endemism rate of up

to 31%, it has been shown that Turkey's Mediterranean region has the highest species diversity. Similar to this, high endemism can also be found in the mountain ranges of Syria and Lebanon. Comparative research has shown that genetic differences are not equally distributed, and coniferous species are more genetically diverse within their own populations in the eastern Mediterranean (Fady & Conord, 2010).

The most important issue threatening the biodiversity of the Mediterranean is ongoing global climate change. In the case of endemics, the risk of population size decline owing to aridization is higher, especially for cold-adapted conifers like *Abies cilicica*. The East Mediterranean mountains, a local hub of endemism in the region, are where the Cilician fir thrives. Because of the species' scattered range, small-size populations are more common. A major decline in the species distribution may have resulted from genetic divergence that was sparked by the preceding glacial cycle, according to earlier studies. (Mayol et al., 2015) As a result, two lineages—*A. cilicica* subsp. *cilicica* and *A. cilicica* subsp. *isaurica* (Turkey)—are recognized at the subspecies level (Turkey, Syria, and Lebanon). The remaining populations of *A. cilicica* may be in danger of extinction due to the effects of impending climatic changes in the East Mediterranean, which are projected to have a significant impact on precipitation and overall warming (Beridze et al., 2021).

### 1.5. Importance of the adaptational common garden (nursery) experiments

When biological forms are exposed to climate change, there are three possible critical responses (Aitken et al., 2008; Parmesan, 2006). First, they can disperse and alter the boundaries of their distribution, controlling the contemporary geographic rangerepartition of their biological specialization; second, they quickly adapt to the unique climatic conditions through (possibly transgenerational) phenotypic versatility; or third, over time, they advance genetically to deal with the unusual climatic conditions through a versatile prepare. Fortunately, common gardens allow researchers to circumvent this issue and measure the total amount of genetic diversity among groups in environments where offspring from different populations are nurtured under the same (sets of) natural conditions (de Villemereuil et al., 2016; Kawecki & Ebert, 2004).

The phrase "adapt, move, or die" sums up the variety of outcomes that can occur from populations assembling novel climate management, but a thorough conceptual foundation for predicting how different plant species would respond to climate change is still lacking (Chevin et al., 2010; Kearney & Porter, 2009; Parmesan & Hanley, 2015).

Common-garden experiments are plantings of species or populations gathered from certain geographic locations and grown together under similar conditions, either indoors or outdoors (Berend et al., 2019). In order to get the greatest possible outcome for population resilience, these studies examine how heritable differences and phenotypic plasticity influence, how populations react to climatic conditions and how they are established by collective choice. The basic concern of the characteristic hypothesis is that tradeoffs govern climate change, or more specifically, that increasing one characteristic's adaptive commitment will compromise another characteristic's support (de Villemereuil et al., 2016; Shipley et al., 2016). In this way, when populations are relocated from their local climate to a different environment, tradeoffs and adaptive optima should become apparent. Currently, the characteristic meetings of relocated populations in new climatic regions do not produce the optimum outcome (Kawecki & Ebert, 2004).

A focused but overwhelming effort is required to investigate the genetic underpinnings of complex traits with polygenic inheritance that are substantially impacted by the environment. Undoubtedly, the influence of various elements, such as genetic fluctuation, phenotypic plasticity, the complicated history of statistics, and genetic recombination, virtually always make it more difficult to find evidence of hereditary fitness. Common garden plant testing is one of the effective tools that developmental scientists have developed to overcome these obstacles in the specific situation of the environmental settings. Using a quantitative genetics tool of heritable traits to develop and consider genotypes from different populations in a shared environment, this type of experiment aims to control the effects of phenotypic variation and, to a certain extent, genotype interactions concerning the environment—the genetic basis of complex traits (for instance, life history and morphological characteristics) (de Villemereuil et al., 2016).

The common garden test is used to test for local adaptive signals in characteristics of interest such as life history characteristics (Kawakami et al., 2011), phenology (Brachi et al., 2013), and growth trait connections because it enables to unwind off the hereditary premise of complex phenotypes over different populations without the confounding effects of the comparing environment (Gonda et al., 2011). Because of the presence of a natural slope, such as aspect (Toräng et al., 2015) or elevation (Alberto et al., 2011), or the proximity of a few distinct circumstances, such as ocean and freshwater (DeFaveri and Merilä, 2014), local adaptation may be suspected. Tests on popular garden plants are used to assess the outcomes of local adaptation for conservation in further detail (Bassar et al., 2010).

Common-garden test designs cover anything from a single garden to a large number of gardens, from a small population to a specific ecotype to a large metapopulation. Comparable transplantation studies, a symmetrical process in which populations and gardens are colocated to decouple immediate climatic consequences from those of developmental climate history, are a particular strategy that research experts like. Since genotype-by-environment interactions (GE) favor genotypes at their local environment relative to nonlocals at the precise place or to genotypes absent from the local environment, the goal is to uncover evidence of local adaptational mechanisms (Kawecki & Ebert, 2004). Common garden plant test strategies can indicate common behaviors under precise climatic change as well as the consistency of evolutionary responses to climate drivers across species, place, and time.

### **CHAPTER 2**

#### LITERATURE REVIEW

#### 2.1. Seed, cone, and seedling studies

The goal of a study was to determine the morphological and physiological traits of the Cilician fir seed. The research lab assessed the 1000-seed weight, the length, width, and thickness of the seeds, as well as the weights of the individual seeds, for each of the five separate provenances that were sampled; the provenances varied in the morphological properties of the seeds. They attempted to germinate the seeds from each of the five different provenance sources after pre-chilling at 4°C for 0, 2, 4, and 6 weeks in order to determine the precise duration of pre-chilling. After three independent pre-chilling times, the seeds from three provenances were put through a germination test at four distinct temperatures each (12, 16, 20, and 24 °C) to determine the ideal germination temperature (2, 4, and 6 weeks). Different germination rates and speeds result in this. At 20 and 24 degrees Celsius, the seeds grew the fastest (Ylmaz and Yüksel, 2014).

In a study where Keskin and Şahin (2000) examined the cone and seed characteristics of Cilician fir, seeds were collected from three different altitudes and ten other experimental areas from the natural distribution areas of the species. The morphological features of the cones and seeds of the species were investigated in detail. This study found that cone and seed characteristics of Cilician fir varied significantly concerning regions, altitudes, subspecies, and years.

A research study on European silver fir (*Abies alba* Mill.) determined the weight of air-dried seeds collected from 4 populations at an altitude of 625-750 m. They defined the survival status of these seeds by x-ray radiography. In addition, they reported

the variation in seed characters such as volume, surface area, wing color, and seed size (Skryszewska and Chlanda, 2009).

Different light and temperature treatments were applied in a study examining how the chilling process affects the germination percentage in firs. In these germination trials, 4% of seeds were subjected to the chilling process at 30 °C for 0 and 20  $^{0}$ C for 9 weeks. Although germination occurred in both treatments, it was found that the germination percentage was 64% in seeds subjected to the chilling process at 20 °C for nine weeks (Tilki, 2004).

The genetic structures of the 7 Kazdağı fir populations in Kazdağları were examined in terms of seedling characteristics. In this study, eight seedling characteristics were examined for two years in the seedlings obtained from the nursery environment. As a result, statistical differences were reported between populations and families within populations (Velioğlu et al., 1999a).

In the study conducted on seed maturity of *Abies pindrow* Spach., it was stated that there was a relationship between humidity and cone maturation, and the average germination percentage in the collected seeds was 32% (Singh, 1998).

In a study, the seeds from the natural distribution area of Kazdağı fir were collected from 124 trees belonging to 4 populations of different sizes and isolation degrees. As a result, it was revealed that Kazdağı fir has a significant genetic variation despite its narrow distribution. The same study aimed to determine the genetic structures of the populations of Kazdağı fir (*Abies equi-trojani* Aschers. Er. Sint.). The results revealed that the average genetic diversity parameters estimated for all the studied populations were slightly lower than those reported for other coniferous species. It was reported that genetic diversity parameters would benefit the conservation programs related to the species (Gülbaba et al., 1996).

In a study to determine the germinating capacity and values of *Abies amabilis* (Dougl.), the seeds were collected from six populations in Forbes, Vancouver Island. It was determined that there were differences between the populations in terms of germination values (Davidson et al., 1990).

According to a research done on two distinct kinds of fir, (*Abies lasiocarpa* (Hook.) Nutt. and *Abies balsamea* (L.) in Canada), the study of the cone and vegetative characters of each tree as a result of morphological and anatomical variation studies, this difference was found among individuals from which the seeds were collected. It is stated that there is significant diversity in terms of characters (Parker et al., 1981).

### 2.2. Adaptive seedling traits

In a study, genetic variations were studied in terms of six populations sampled from the natural distribution areas of Cilician fir and seedlings belonging to 150 families, 25 in each population and 1+0 old seedling characters. Heritability at the individual and family levels, some genetic parameters, and genetic and phenotypic correlations between characters were also evaluated in this context. With the exception of epicotyl height, the results indicated significant variations in the traits of one-yearold seedlings among populations and families within populations Additionally, based on the seedling and seedling characteristics examined, estimates of medium and high individual and family heritability were reported. As a result, it can be said that in-population family selection is more important than population selection in breeding studies to be carried out in this species (Gülcü and Dirlik, 2016). However, the study is limited to only one year old seedlings.

Another study discussed suitable shading for this species, two different planting seasons, and planting density problems for growing Cilician fir and nursery conditions. The most appropriate combinations for growth of this species were determined. In addition, some seedling characteristics of (4+0) old Cilician fir seedlings grown in spring, autumn, shady, unshaded, and different planting density environments were determined, and the seedling characteristics of these seedlings were compared. It was determined that two other planting dates, shading and density, influenced the seedling survival percentage. The seedlings with the highest survival percentage were those grown in spring shade and with a planting density of 150-200 seedlings per square meter (Gökdemir and Kızmaz, 1998).

Three Turkish and two Trojan fir populations were studied to determine their origin features, needle and cone traits, and seed germination potential. Highlights of the provenance (vigor score, crown score, and color) and mother tree features (size lesson, breast height, and tallness) were remarkably identical across species and within species. Turkish fir's needles were overall (p 0.05) longer and wider than Trojan fir's. Additionally, compared to Trojan fir, Turkish fir had a wider cone and a better cone width to length ratio. Relationships between needle-cone features and area parameters were direct, favorable, and crucial (rise, scope, and longitude). Along an altitudinal angle, the needle estimate extended northwards, eastwards, and upwards (Kurt et al., 2016).

In another study carried out in Kazdağı fir (*Abies equi-trojani* Aschers. et. Sint.), seeds collected from a total of 26 families sampled from 4 populations were used. In the grown seedlings, characteristics such as cotyledon number, hypocotyl length, number of buds, lateral branches, seedling height, and survival were examined. The family heritability varies between 0.03 and 0.37, and the Çan population is defined as the genetically distant from other populations. It is stated that the genetic correlations of the observed seedling characters and the phenotypic correlations are in the same direction and higher than latter. (Velioğlu et al., 1999c).

In a study conducted on *Abies procera* Rehd., it is stated that 25% of the variation in the number of cotyledons and 45% of the variation in seed weight differ from year to year, and there is no relationship between the number of cotyledons and seed weight within the family (Sorensen and Franklin 1977).

A study to reveal the genetic variation pattern in *Abies sachalinensis* was conducted to determine the differences between the populations by measuring cone length, seed weight, germination percentage, cotyledon number, and height and diameter development for four years in 117 families sampled from 7 populations. The population-based thousand-grain weight of the seed ranged from 9.3 g to 12.3 g. (Okada et al., 1973).

### **2.3.** Genetic studies (Molecular and morphological traits)

Beridze et al. (2021) utilized the Bayesian approach to examine endemic *A. cilicica*. Mainly, they assessed the likely time of the dissimilarity of intraspecies to confirm past presumptions of the species' evolutionary history. Moreover, speciation modeling was utilized to lay out the potential routes of changes within the past and to show cold refugia in which the species continued to strive in a climate emergency. This method was used to look at how the distribution of *A. cilicica* populations would change in the future due to climate change. Their findings showed that the Riss glaciation, which occurred around 220 ka BP, coincided with the discrepancy between the populations of Lebanon and Turkey.

For in situ gene preservation programs for fir species, as well as for determining the genetic similarity between populations of the *Abies equitrojani* (Asch. & Sint. ex Boiss) Mattf. species, which is unique to Turkey, and the *Abies nordmanniana* species complex, Kaya et al. (2008) suggested evaluating the usefulness of RAPD (randomly amplified polymorphic DNA) and cpSSR (simple sequence They discovered

that the template DNA pooling for RAPD or cpSSR marker screening of the fir populations may be successfully used to expedite genetic conservation and taxonomic research. In the PCR (polymerase chain reaction) mixture, it is advised that DNA pooling for layout DNA be limited to 7 megs, but at the very least, five replications test different sets of families each time.

In order to find proof of sizable genetic differentiation, Sekiewicz et al. (2015) performed a genetic analysis of nuclear microsatellites in *Abies cilicica* populations growing in the West Taurus, East Taurus, and Lebanon mountains. Despite range fragmentation, all of the populations under study nevertheless exhibited considerable genetic differentiation ( $F_{ST}$ = 0.14) and a relatively high degree of gene diversity (He= 0.724), traits typically found in Mediterranean tree species. Significant inbreeding, the bottleneck effect, and small effective population sizes were found, though, and these factors may have a deleterious impact on the species' genetic resources. Three distinct clusters—"West Taurus," "East Taurus," and "Lebanon"—that match the geographic makeup of the species' native range were discovered by Bayesian cluster analysis. Lebanese populations of *A. cilicica* showed some genetic mixing with East Taurus populations, but not with West Taurus populations. They argue that notable observed genetic isolation between areas of species event is likely caused by the long-lasting geneic confinement that started there along with the Pleistocene climatic rotation and was subsequently strengthened by human-induced deforestation.

Çiçek et al. (2005) grew seedlings of 126 families from four natural populations of Kazdagi fir (*Abies equitrajani*) in Turkey in nurseries and assessed them for 12 adaptive traits to determine the extent and pattern of genetic variation in these variables (including growth and phenological traits). For the most investigated traits, significant population and family within population differences were identified. Despite the fact that the components of total variation attributable to families within populations were larger than those attributable to populations, the proportions of variance

attributable to populations (ranging from 0-5.7%) and families (trees) within populations (ranging from 0-20.7%) were both small.

A study surveyed the effect of demographic and developmental forms on population genetic differences and structure utilizing ten nuclear microsatellite loci. All 15 nearby populations uncovered a low regeneration but an exceedingly successful population establishment. Low regional genetic structure was detected using  $F_{ST}$ -based estimates of population genetic differentiation, but a crucial Northeast-Southwest population structure was identified using Bayesian analysis of population structure. Populations displayed non-equilibrium conditions between dispersal and genetic drift, seeming effective, but weak in isolation by distance. A deviated Northeast-Southwest relocation, including a few long-distance dispersal occasions, was identified by Bayesian assignment tests. They suggest that at least two demographic processes during its recent evolutionary history contributed to the persistence and Northeast-Southwest geographic structure of *Abies cilicica* in Lebanon: (a) recent migration to presently marginal populations, and (b) local persistence through altitudinal shifts along mountainous geography. The importance and usefulness of their findings to species response to climate change were further discussed (Awad et al. 2014).

*Abies cilicica*, which spreads between 1200-2000 meters and forms natural forest stands, was chosen as a target species for *in-situ* studies due to its robust demands and various problems at the beginning of afforestation. On the endosperms of open-pollinated seeds gathered from 200 trees in four populations, isoenzyme analyses were done. In seven seeds from each tree, 15 enzyme systems were studied, and 24 loci were determined. Twenty-three of these loci were found o be polymorphic. In total, 46 alleles were found at the loci under study. In the analysis made, it was determined that an allele that could not be detected in one population or that was rare in the other populations. It is suggested that the results were an expected consequence of genetic drift in isolated and small populations. According to the data, it can be

said that Cilician fir harbors a large amount of genetic diversity in its populations (Özer, 2000).

In the Kovada lake forest district of Isparta, Turkey, a study was conducted to evaluate the rates of tree growth (basal area incrementrom recently dead and living Cilician fir (Abies cilicica Carr.)) trees. In the study area (11 test plots), tree growth rates were examined in 2006 to determine whether any deceased trees had pre-death growth depressions. They contrasted the size and growth rate of the dying trees to a control group of still-living trees. Compared to control trees, basal area increment (BAI) was much lower on average throughout the final ten years before death. On average, 50–60 years prior to death, trees started to develop differently. Approximately 18 percent of dying trees had consistently modest growth, 46 percent showed a clear drop in growth, and 36 percent had healthy growth up to their demise. Furthermore, 12 mortality models were established by comparing the tree-ring-based growth patterns of dead and surviving Cilician fir trees. It made use of logistic regression with tree-ring growth patterns as the indicator variables. The four models that performed the best overall correctly identified 43.8-56.3 percent of all dead trees and 75.0-87.5 percent of all surviving trees. They also predicted that 25.0-43.8 percent of all dead trees would die within a range of 0 to 15 years before their actual death year (Carus, 2010).

A study aimed to determine the phylogenetic relationships between the fir taxa distributed in Turkey by core DNA ITS sequence analysis and to develop in situ conservation strategies of the species by revealing the genetic diversity at the population level with microsatellite markers. For this purpose, Sampling was made from 16 populations determined to represent six fir taxa distributed in Turkey. Observed heterozygosity (Ho) values used to express genetic diversity according to the results of microsatellite analysis. It was 0.44 for *A. nordmanniana* subsp. *nordmanniana*, 0.45 for *A. nordmanniana* subsp. *bornmuelleriana*, 0.37 for *A. nordmanniana* subsp. *equi-trojani*, 0.35 for A. *cilicica* subsp. *isaurica*, 0.40 for *A. cilicica* subsp. *cilicica* 

and 0.34 for *Abies x olcayana*.  $F_{st}$  value of 0.15, showing the distribution of genetic diversity among populations, was calculated. According to this result, the genetic diversity among the populations was 15%, and the genetic diversity within the populations is 85%. They stated that the high genetic diversity within the populations is also an indicator of the high genetic diversity of the species (Tayanç et al., 2013).

Turna et al. (2010) selected 12 populations and 20 trees (families) in each population to reveal the genetic diversity depending on some morphological characters in Uludağ fir. In the seeds taken from the trees in the trial area, nine morphological features were determined, including seed length, width and weight, carpel length and weight, wing width, and length. In the study, it was revealed that there are morphological differences between these populations.

Seedlings obtained from the seeds collected from 4 natural populations from the natural Kazdağı fir in Kazdağları were examined. Because of the study, it was determined that the within-population variance was higher than the between-population variance. In addition, it was determined that the genetic correlation of seedling characteristics was in the same direction as the phenotypic correlations (Velioğlu et al. 1999b).

A postgraduate thesis study named "Biosystematic Researches on the Species of *Abies Miller*. in Turkey" aimed to determine the taxonomic boundaries of 5 subspecies belonging to two species and the natural variations between subspecies. Morphological properties and essential oil components were subjected to statistical analysis. According to the results of the analysis, it was found that the variation within and between the populations of the subspecies was high. It was also revealed that essential oil components quantitatively varied between subspecies and populations. Although taxa are very similar to each other morphologically and chemically, it has been determined that some characters can be used reliably (Bağcı, 1998).

In a study carried out to reveal the genetic diversity in natural Anatolian black pine populations separated as seed stands by the Ministry of Forestry and Water Affairs, it was concluded that these seed stands are genetically different from each other and that a large part of the resulting genetic diversity stems from the differences between trees in the stand (Kaya and Temerit, 1994).

#### 2.4. Provenance and progeny tests

By using a new method, information from advanced age provenance testing was reexamined in order to directly assess the development of populations at their particular destinations under independently produced future climates. The results revealed that fir species have a high degree of adaptability. At the xeric boundaries, insulators are investigated regarding the development and survival of silver fir under potential climate situations. In order to examine the prospects of adaptable silviculture, help exchange silver fir populations, and present non-autochthonous species, the distinctive signature of previous climate dictating the current and anticipated development was taken into consideration. Hargreaves' climatic moisture deficit was chosen to illustrate how adult populations react to tallness. The rather dry season stretch of the test location's populations was surveyed using climatic exchange separate, and these results were compared to the previous conditions to which the populations had adapted. Past, present, and future moisture shortage circumstances were solely determined by ClimateEU and ClimateWNA pathway RCP8.5 information. Other fir species from South Europe and the American Northwest were also tested in addition to the silver fir (Matyas et al., 2021).

In a research study carried out in *Abies sachalinensis*, the seasonal variation of cone weight and chemical structure in cones collected from different clones found in the seed orchard was examined, and the weight of the cones was found to increase from 160 mg in May to 12,300 mg in the fall, with a gradual weight increase. In addition,

it reported a strong relationship between chemical substances and germination rate (Ujiie et al., 1991).

A study in *Abies grandis* Lindl. compared the growth and drought response of seedlings grown from seeds collected from 43 populations. It was discovered that populations varied significantly in terms of branch drying rates, mortality after planting, needle spotting, height growth, and damage from late frosts (Scholz and Stephan 1982).

# 2.5. Breeding and conservation studies

In a recent study, the importance of the recently discovered population of Cilician fir, which is closely located in the steppe climatic region, was discussed for its habitats and characteristics as a fir stand. This new growing area of Cilician fir is located in the range of  $38^{0}07'27"-35^{0}13'59"$  and ,  $38^{0}07'52"-35^{0}15'40"$  N, and  $38^{0}08'19"-35^{0}15'13"$  E. This may be an important genetic resource of the species (Keleş et al., 2012).

A new variety of Cilician fir from Anatolia was described in another study. Herbarium examples of this variety (*Abies cilicica* Carr. subsp. *isaurica* Coode and Cullen var. pyramidalis Boydak and Erdoğrul) were collected and kept within the herbarium of ISTO (Herbarium of Faculty of Forestry, University of İstanbul) together with their photos. Measures were taken to clarify the modern assortment (Boydak and Erdoğrul, 1999).

A research study on Cilician fir (*Abies cilicica* Carr.) in the Seydişehir forestry district aimed to contribute to silviculture and genetic breeding of the species in terms of cone yield and growth characteristics (height, breast diameter, crown diameter, and age) and relation to elevation. Because of the study, there were differences between and within the population regarding cone yield. Based on results from correlation analysis, there was no statistically significant relationship between height

and cone yield. However, a statistically significant difference was found between breast diameters, age, height, and crown diameter (Ülküdür et al., 2013).

On the other hand, under the coordination of the Research Institute for Forest Trees and Seeds under the General Directorate of Forestry (GDF), the nationwide followup of seed stands, seed orchards and gene conservation forests, and progeny test projects are carried out. Seed stands are the ones that are selected as a seed source and operated with a particular silviculture plan because they consist of trees with superior characteristics in terms of wood properties, which are economically important in the ecosystem. Seed stands constitute the beginning of improvement studies and function as an indispensable seed source until sufficient seeds are produced from seed orchards. In addition, since most of the selected seed stands are the best examples of natural stands, they constitute the most crucial part of our forest gene resources. Gene conservation forests are the ones that are selected and managed to protect the genetic diversity of a species. With gene conservation forests, it is aimed to protect the genetic richness existing in nature and transfer it to future generations. In order to produce high-quality, abundant, dense, and easy seeds, clonal seed orchards are established with grafted seedlings copied from seed stands or plus trees selected from gene protection forests. It is possible to produce 8% more per unit area than seed stands in plantations to be made with seeds produced from seed orchards. Progeny tests reveal superior genetics of phenotypically selected plus trees whether they have these characteristics. According to the results of the progeny tests, it is possible to provide 25% more production than the unit area in the afforestation to be made with the seeds produced from the genotypic seed orchards to be established (GDF, 2014).

# **2.6.** Climate change and the future of Turkish firs concerning adaptation to changes in temperature and precipitation

The anticipated temperature rise, precipitation diminishes, and concentration of precipitation in extraordinary occasions seem to initiate developmental decay and dieoff on tree populations found at the topographical dispersion constrain of the species. Understanding versatile capacity and territorial helplessness to climate change in Mediterranean forest lands are not well understood and requires more focused investigation endeavors (Linares et al., 2011).

With the exception of *A. numidica* and *A. pinsapo*, all fir species were taken into consideration in Aussenac et al. (2002) which found that a conceivable increase in temperature without an increase in precipitation would create a high risk that the display zones of the circum-Mediterranean would diminish for all fir species within minor zones of their ranges, but also in other zones with a southerly angle and exceptionally shallow soils. There is a higher likelihood that *A. cephalonica* and *A. cilicica*, species with early bud burst, will be harmed by late frosts in expansion to the effects of water stretch. However, the other species may be impacted by *A. nordmanniana* and *A. bornmulleriana*, albeit to a lesser extent.

A study emphasized increment and growth relationships in different old-growth fir forests in the Mediterranean region. Relationships are based on measurements of 224 sample trees from various sites. In the site designation of the sample points, the relationship between the site grade and the site curves representing the average stand height curves was established. The diameter-diameter increase relationship followed as a bell curve in Cilician fir stands of different ages. However, the diameter-diameter increment points on the graph are scattered, which is thought to be caused by measurement errors, neighborhood relations between trees, and climate changes. In addition, it was observed that the multiple coefficients of determination of the Diameter-Diameter Increment-Bonitet degree relationship were also very weak (Yıldızbakan and Saraçoğlu, 2004).

In a study, the volume relations to being in pure and mixed stands of Cilician firs, which form partially pure, but mainly mixed stands in the high altitudes of Taurus and Amanos Mountains, were investigated. For this purpose, 10 sample areas were taken, four in pure Cilician fir stands, two in fir-Anatolian black pine mixed stands, and 4 in fir-Taurus cedar mixed stands. Stand profiles were drawn in the sample

areas, and the existing tree health was determined. Thus, the volume relations in pure and mixed stands of Cilician fir were revealed. Although the sample areas were taken from the parts of pure and mixed fir stands showing normal establishments as much as possible, irregularities in the past in almost all areas or traces of sections far from the technique were found. Therefore, the determined establishments reflect "actual establishments", not "optimal". Pure stands of Cilician fir, generally of different ages, show irregular establishments. Therefore, tree health and increment per hectare decrease. To increase the volume per hectare, these stands should be brought into consideration of the institutions required by the revenue according to the site (Bozkuş,1997).

Another research determined the natural distribution areas of Cilician fir in Turkey and detailed research about the needle leaves, cones, shoots, ecological demands, and silvicultural characteristics of the species. It also provided information about the species' seeds' morphological characteristics (1000-grain weight). It has been determined that the seeds of this species have the highest 1000-grain weight among other domestic fir species (Bozkuş,1988).

Aslan (1982), in his study on the nursery technique of Kazdağı fir (*Abies equi-tro-jan*), determined that this species has a thousand-seed weight of 55.33 g, sowing time is early spring or autumn. It is recommended that the planting depth should be 1-2 cm, and the covering material should be forest soil. He stated that shading should be done for this species.

# **2.7. Justification of the study**

If future climate change scenarios pan out as expected, this will result in (1) an increase in annual temperature (2-6 °C), (2) a decrease in annual precipitation (20%), (3) seasonal variation in both temperature and precipitation, and (4) regional variation in both temperature and precipitation (Önol and Semazzi 2009). The Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC 2007)

based on the outputs of the Global Circulation Model (GCM) studied for various emission scenarios predicts that Turkey and the Mediterranean Basin will experience significant decreases in precipitation with rising temperatures by the end of the twenty-first century. In light of this, it has been predicted that this area will be among the most vulnerable to climate change.

A reasonably extensive natural range of 3,400 km<sup>2</sup> has been estimated for *Abies ci*licica in Turkey, Lebanon, and Syria. At the national level, the limited subpopulations of the common subspecies in Lebanon and Syria should be regarded as extremely endangered due to their severe degradation. Southern Turkey's Mediterranean vegetation, especially in the mountainous areas, faces a significant risk from climate change. Summer time temperatures have increased, and annual precipitation has decreased over the past 50 years, according to climate data records. These patterns are increasing the likelihood of fire while also causing the general health of the trees to decline, leaving them more open to disease attack. The number of tourists in the Taurus Mountains is also significantly increasing, enhancing the risk of forest fires (Öztürk 2010). If these unfavorable trends continue, this species might meet the requirements to be classified as vulnerable under IUCN danger category B2 (Gardner, 2013). In any case, at this arrangement, an appraisal of close undermined superior reflects its moderately constrained zone of inhibitions and the current degree of the decline. Moreover, this means that the range of Abies cilicica will change. It will either move up in elevation about 200 meters or 2 degrees of latitude to the north. (150 km north).

From the previous studies, there is not enough genetic study to date that addressed genetic diversity patterns in adaptive seedling traits. Thus, the adaptive seedling common garden experiment is required to evaluate the extent and pattern of genetic variability in seed and adaptive seedling traits and to comprehend the survival and growth of a wide range of sampled populations in a shared habitat. This information may be helpful for foresters to efficiently design assisted migration and genetic resource conservation programs dealing with the Cilician fir.

# 2.8. Objectives of the study

Knowing the extent and pattern of genetic variation in adaptive seedling traits like growth and phenological traits in Cilician fir (*Abies cilicica*) populations representing the natural range of the species will be useful in light of expectations for climate change, increased forest fires, and anthropogenic factors.

In order to test whether the pattern of genetic variation has adaptive significance, the goals of the current study were thus set as follows: (1) to determine the pattern and magnitude of genetic variation in growth and phenological seedling traits; (2) to estimate genetic parameters for growth and phenological traits; and (3) to ascertain the relationship between seed and seedling traits as well as their relation with topographical variables.

# **CHAPTER 3**

#### **MATERIALS & METHODS**

#### **3.1. Selection and sampling of populations**

In sampling of populations, the species' natural distribution area represented as much possible. In Turkey, Abies cilicica species was classified as two taxa and six significant areas with the populations of Yenişarbademli, Akseki, Ugurlu, Cennetdere, Goksun, and Tufanbeyli by Tayanç et al. (2013). Using the diversity pattern indicated in Tayanç et al. (2013) and adding two of Develi and Andırın as extreme populations, 8 populations were determined for sampling mother trees (families) for cone and seed collections. The geographic and topographic information about sampled populations was provided in Figure 3.1 and Table 3.1. The mother trees from which the cones were collected, were selected from healthy and close-aged trees in the overstory of the stand, not-stressed and representative of Cilician fir stands. To get open pollinated (half-sib families) seeds, 20 mother trees (families) meeting the above conditions were identified for cones collection from the mother trees from selected populations. The cones were hand-picked from September to October 2018. The collected cones were transported to the Central Anatolia Forestry Research Institute Behiçbey Nursery facilities in Ankara. They were subjected to watering and drying processes to facilitate the cones' cracking in getting seeds out. During cone collection and mother tree selection within each population, the following additional rules were applied: (i) collection of cones from the upper 1/3 tree crown, (ii) ( at least 100 m distance between families (mother trees), (iii) there should not be an elevation difference of more than 300 m between families, (iv) ensuring that the families were about the same age.

Each mother tree (family) was coded with a number, coordinates, and altitude data. The collected cones were placed in a separate bag for each tree. Cones were dried in baskets at the greenhouse, and seeds were removed on a family basis. Seeds were bagged and labeled on a family basis and stored in cold storage at  $+4^{0}$ C. In addition, the populations were coded for convenience in the following steps (Table 3.1.).



Figure 3.1. Location of studied populations in Turkey (Google Earth, 2022)

# **3.2. Experimental Design**

The seeds planned to be planted in February-March 2019 were planted in December 2018. The reason for this is the empirical evaluation that the winter conditions of Cilician fir seeds under the ground will increase germination, according to the results obtained from the germination studies carried out with nursery experts working with the Cilician fir in the previous years in the Olcay Forest Nursery in Konya and the study of Yılmaz and Yüksel (2014).

Two stages comprised the nursery experiments:

A random block design with two replications was utilized in the experiment's initial phase to produce enough seedlings for the common garden nursery experiment. Open-pollinated seeds from 160 families of eight populations sampled from

the species' natural range were sown in plastic tubes filled with nursery soil as three seeds per tube in December 2018. For each family, there were 50 tubes, 25 of which were in each replication, so there could be sufficient seedlings to be used in the second phase of the experiment. In this stage, mean germination percentages, cotyledon numbers, and mean survival rate was recorded (Table 4.4). In addition, seed width, thickness, and length of the populations were measured by Milton micrometer (Table 4.1). The definition of seed and seedling traits and their units are provided in Table 3.2.

Table 3.1. Description of the studied populations. Temperature and precipitation values were taken from State Meteorological Service (SMS).

Population	Taxon	Code	Latitude	Longitude	Altitude	As- pect	Annual Mean Temperature (SMS 1991- 2020)	Annual Mean Precipitation (SMS 1991- 2020)
Akseki/ANTALYA	subsp. isaurica	AKS	37°06'51"N	31°46'52"E	1350	NE	18.8	1060.0
Bucak/BURDUR	subsp. i <i>saurica</i>	UGU	37°19'52"N	30°37'41"E	1200	NW	13.3	426.9
Yenişarbademli/ISPARTA	subsp. isaurica	KIZ	37°45'52"N	31°22'00"E	1400	NE	12.3	568.4
Tarsus/MERSİN	subsp. cilicica	TAR	37°07'22"N	34°29'05"E	1370	N	19.2	613.9
Tufanbeyli/ADANA	subsp. cilicica	FEK	37°47'58"N	35°56'58"E	1400	N	19.2	668.7
Göksun/KAHRAMANMARAŞ	subsp. cilicica	GÖK	37°48'35"N	36°21'17"E	1700	NW	16.7	721.6
Andırın/KAHRAMANMARAŞ	subsp. cilicica	AND	37°38'44"N	36°20'06"E	1400	NE	16.7	721.6
Develi/KAYSERİ	subsp. cilicica	DEV	38°20'50"	36°04'10"	1680	NE	10.7	389.6

Since 52% of the seedlings in the first part of the experiment did not survive, the experimental design was changed for the second phase, which began in March 2020. On four seedling-row plots totaling 160 families, open-pollinated seeds from parent

trees were put in a forest nursery bed at the Olcay Forest Nursery in Konya. A complete block design with six replications was used to map the placement for a total of 3840 seedlings (4 seedlings  $\times$  20 families x 8 population x 6 replicates) in the spring of 2020 (24 seedlings per family in total) (Figure 3.2). The Olcay Forest Nursery was used as a nursery is in the Meram district (latitude 37° 49' 22" and longitude 32°25'26"), almost 21 km from Konya's city center. The altitude of the nursery is about 1016 m. The annual mean temperature in Olcay forest nursery is 11.5°C and the annual mean precipitation in Meram is 327 mm/year.

Fir species are tree species that need shelter in their early seedling stages. For this reason, after planting, in order to protect the seedlings from the direct effect of the sun, to create conditions close to their natural environment, and to increase the germination rate, a shield was created using 50 cm high tulle with 30% light transmittance (70% shielding) of the planting nursery beds. The shielding process was carried out in the nursery conditions from June-August of 2020, when the air temperatures were high. Thirteen traits expressing seed and seedling traits include survival, height increments, diameter growth, and phenological traits during the 2020 growing season (Table 3.2.).

# **BLOCK I**

**BLOCK II** 

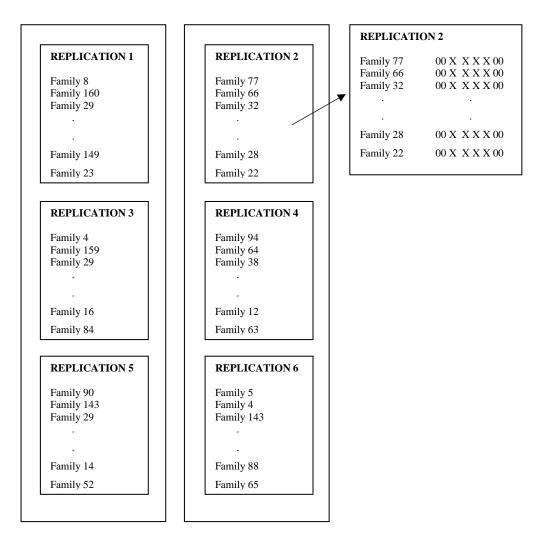


Figure 3.2. Nursery design and layout of the experimental seedlings

Codes for the studied traits	Definition of traits	Units of traits		
Seed traits				
SW	Weight of 1000 seeds	gram		
SeedW	Width of seeds	mm		
SeedT	Thickness of seeds	mm		
SeedL	Length of seeds	mm		
Seedling traits				
Germ (%)	Seedlings germinated	%		
CotNum	Cotyledon numbers	number		
Ht19	Height increment in 2019	cm		
BB20	Budburst data in 2020	Number of days from January 1, 2020		
SURV184	Survival recorded on the 184 <sup>th</sup> day of the year 2020	%		
BS20	Bud set in 2020	Number of days from January 1, 2020		
Surv364	Survival recorded on the 364 <sup>th</sup> day of the year 2020	%		
Ht20	Height increment in 2020	cm		
Diam20	Diameter growth measured in 2020 (increment of 2019 included)	cm		

Table 3.2. Description of studied traits

#### 3.3. Statistical analysis

Genetic correlations were estimated from components of variance and covariance (Falconer, 1981) and substituted into the standard equation for the product-moment correlation coefficient. Heritabilities were estimated from components of variance. Analyses of traits were based on plot means. In each replication, there were some plots with no seedlings in family plots; thus, variance analysis was carried out using a generalized least square procedure (SAS Institute, 2018). A GLM procedure of SAS was used, which gives unbiased estimates of all mean squares when a data set has missing plots. The following statistical model has been used during the data analysis.

 $Zijk=\mu + Bk + Pj + F(p)i + eijk$ 

where  $\mu$  is the experimental mean, Zijk is the mean performance of *i* th family in the *j* th population in the *k* th replication; Bk = the effects of replication; Pj = the fixed effects of populations; F(p)i = the random effects of families within populations;

eijk = the experimental error.

According to the predictions from the analysis of variances, the variance and covariance components of populations and the families within populations were estimated. (Table 3.3.). Similar to Namkoong (1979), heritabilities were determined using variance components. The following equation was used to estimate family heritabilities  $(h^2_{(fx)})$ :

$$h^{2}_{(fx)} = \sigma^{2}_{f(x)} / (\sigma^{2}_{e} / r + \sigma^{2}_{f(x)})$$

where  $\sigma_{f(x)}^2$  = family component of total variance for trait *x*, r = 4.77,  $\sigma_e^2$  = error variance. Genetic correlations were estimated from the component of variance and covariance (Falconer, 1981) and substituted into the standard equation for the product-moment correlation coefficient.

Genetic correlation

$$\mathbf{r}_{\mathbf{g}(\mathbf{x},\mathbf{y})} = COV_{f(x,y)} / (\sqrt{\sigma} \, {}^2_{f(x)} \sqrt{\sigma} \, {}^2_{f(y)})$$

where  $r_{g(x,y)}$  = estimated genetic correlation between trait *x* and *y*,  $\sigma^{2}_{f(x)}$  = estimated components of variance of families within populations for trait *x*,  $\sigma^{2} f(y)$  and = estimated components of variance of families within populations for trait *y* and  $COV_{f(x,y)}$  = estimated component of covariance of families within populations between traits *x* and *y*, estimated from covariance analysis. According to Kaya et al. (1989), family mean squares and mean cross products for the characteristics were used to calculate the phenotypic correlation between traits x and y. According to Becker (1984), the standard errors of genetic and phenotypic correlations were estimated.

Table 3.3. Form of analysis of variance for the traits.

The effect of the population is fixed, and the others are random. (ANOVA table with mean squares was constructed according to this model)

Source of variation	Degrees of freedom	Expected mean squares
Replications	5	$\sigma^2_{e+}$ 4.77 $\sigma^2_{F(P)}$ + 118.6 $\sigma^2_{r}$
Populations	7	$\sigma^2_{e+}$ 4.77 $\sigma^2_{F(P)+}$ 87.7 $K^2_P$
Families/ Populations	141	$\sigma^{2}_{e+}$ 4.77 $\sigma^{2}_{F(P)}$
Error	559	σ <sup>2</sup> e

The raw measurements that make up all the data and the documents containing the estimations based on these data are kept within the General Directorate of Forestry.

# **CHAPTER 4**

#### RESULTS

### 4.1. Pattern and Magnitude of Genetic Variation

# 4.1.1. Seed-related traits

Seed traits such as seed weight, length, width, and thickness varied among populations (Table 4.1). There were also significant correlations between these traits and topographical traits. There were strong and negative associations between characteristics of seeds and latitude and altitude. The populations from the south to northern latitudes or low altitude to high altitude had smaller values of seed-related traits (Table 4.2). For example, the families from high latitude and altitude had less seed weight, thickness, and width than those families originated from low latitude and altitudes. The seed-related traits had significant positive correlations with growth traits. Those families or populations with larger seed mass (weight-length-widththickness) had higher height growth in 2019 and 2020 and diameter growth in 2020 compared to those with smaller seed mass. However, the families of populations with heavy seeds had negative correlations with the budset timing in the growing season of 2020 (Table 4.3).

# 4.1.2. Seedling traits

#### 4.1.2.1. Germination, Cotyledon numbers, and survivals

In general, germination and survival were low in studied populations. Nevertheless, there were significant variations in the mean percent of seed germination, survival, and cotyledon numbers among populations. The UGU (Bucak/BURDUR) population had the highest percentage of germination (36.6%), while the TAR (Tarsus/MERSİN) population had the lowest germination percentage (4.8%). %). In

Topographical variables	Akseki (AKS)	Andırın (AND)	Develi (DEV)	Tufanbeyli (FEK)	Göksun (GÖK)	Yenişarba- demli (KIZ)	Tarsus (TAR)	Bucak (UGU)
Latitude	37.08±0.003	37.616±0.02	38.33±0.00 6	37.77±0.005	37.71±0.0 05	37.75±0	37.09±0	37.32±0
Longitude	31.84±0.009	36.310±0	36.07±0.00 5	36.03±0.005	36.55±0.0 04	31.38±0.0 02	34.62±0.0 03	30.61±0.0 02
Altitude	1426.25±10 9.57	1748.00±118.6 3	1817.55±66 .82	1578.45±50.43	1434.60±7 0.86	1267.45±1 5.47	1255.50±1 8.76	1224.45±2 8.96
Seed traits								
SW1000	12.41±4.06	10.61±2.14	10.51±2.40	12.01±2.22	10.85±2.4 0	18.25±4.7 7	15.83±2.6 4	17.74±2.9 9
Seed-width	6.93±0.46	6.58±0.61	5.89±2.57	7.09±0.52	7.15±0.48	7.17±0.37	7.22±0.48	7.16±0.42
Seed-thickness	4.27±0.37	3.94±0.29	3.28±1.46	4.04±0.27	4.12±0.21	4.95±0.44	4.36±0.29	4.70±0.19
Seed-length	13.47±1.17	13.31±1.03	11.05±4.85	13.51±0.82	12.94±0.7 9	14.75±0.9 5	14.63±1.1 76	14.64±0.7 5
Seedling traits								
CotNum	7.37±0.38	7.34±0.48	7.39±0.29	7.34±0.23	7.33±6.80	7.26±0.19	8.04±0.67	7.51±0.29
Germ%	19.4±13.6	11.65±16.22	30.76±19.9	34.1±15.05	30.7±15.9 6	35.26±14. 9	4.8±9.168	36.6±13.4 6
HT19	19.40±13.64	11.63±16.22	30.77±19.9 7	42.03±14.31	30.70±5.3 3	35.30±12. 95	4.80±9.16	36.60±13. 47
BB20	153.00±0	153.00±0	153.0±0	153.00±0	146.53±31 .01	153.00±0	153.00±0	153.00±0
SURV184	0.965±0.13	0.99±0.05	1.00±0	1.00±0	0.94±0.17	1.00±0	1.00±0	0.99±0.05
BS20	251.39±17.0 7	249.43±8.14	249.55±11. 68	254.08±15.46	257.25±20 .00	251.73±12 .78	249.06±6. 99	250.78±9. 65
Surv364	0.98±0.093	0.997±0.027	1.00±0	0.99±0.05	1.00±0	0.99±0.04 4	0.99±0.03	0.99±0.05
Ht20	47.92±11.78	52.997±10.77	61.87±10.4 0	39.51±11.93	38.60±10. 98	40.38±10. 06	51.58±8.4 0	41.36±9.4 5
Diam20	3.978±0.95	4.13±0.81	4.07±0.73	3.62±0.77	3.66±1.06	3.93±0.95	4.02±0.74	3.80±0.82

Table 4.1. *Population means and standard deviations for the topographic variables, seed, and seedling traits* 

survival percentages, the FEK (Tufanbeyli/ADANA) had the highest survival value (% 42.03). On the other hand, the TAR (Tarsus/MERSIN) population had the lowest survival again (3.0 %). The TAR (Tarsus/Mersin) population was among the worst performers concerning the percentage of seed germination and survival. Populations also varied in the average number of cotyledons. It ranged from 7.26 in KIZ (Yenişarbademli/ISPARTA) to 8.58 in AND (Andırın/KAHRAMANMARAŞ) populations (Table 4.4).

	Latitude	Longitude	Altitude	
Seed traits				
SW	-0.27**	-0.56**	-0.54**	
SeedW	-0.26**	-0.16ns	-0.29**	
SeedT	-0.36**	-0.48**	-0.51**	
SeedL	-0.37**	-0.32**	-0.37**	
Seedling traits				
Germ(%)	0.32**	-0.02ns	-0.13ns	
CotNum	-0.21**	-0.34**	-0.37**	
Ht19	-0.32**	-0.10ns	-0.02ns	
BB20	-0.001	-0.11 ns	-0.21**	
SURV184	0.07 ns	-0.14 ns	-0.21**	
BS20	0.05 ns	0.25**	0.28**	
Surv364	0.05 ns	-0.07 ns	0.04 ns	
Ht20	-0.42**	-0.73**	-0.58**	
Diam20	-0.16 ns	-0.32**	-0.30**	

Table 4.2. Correlation coefficients between topographical variables and seed-seed-ling traits (N=160)

ns - not significant; \* - significant at p < 0.05 level; \*\*- significant at p < 0.01 level

#### 4.1.3. Phenological Traits

Bud burst and budset time in 2020 (BB20 and BS20, respectively) varied significantly among populations and families. In all traits, the portion of total variance attributable to families was significantly higher (12.4 percent in BS20 and 63.6 percent in BB20) than the portion attributable to populations (2.2 percent in BS20 and 5.9 percent in BB20) (Table 4.5). In general, families originating from the low longitude and altitude families had early bud set but later bud burst timings in 2020 than those families from high longitude and altitude families. By way of illustration, families from low elevation populations finished the bud set around one week earlier than those from high elevation populations (Table 4.1).

# 4.1.4. Growth traits

Height increments in 2019 were significantly different among populations. FEK and UGU populations had the highest height increments, while the population TAR had the small height increment (Table 4.1). Since the height increment was recorded in

2019 in the first phase of the experiment, the analysis of variance could not be performed. However, there were major differences in the height increment in 2020 (HT20) and diameter growth in 2020, including diameter increase in 2019. In HT20, the population-related component of total variance (VC = 41.5%) was greater than the family-related component (VC = 14.4%). On the other hand, the part of the total variation in Diam20 that was attributable to families (21.5%) was higher than the part attributable to populations (0.3%). (Table 4.5).

#### 4.2 Family heritabilities and genetic correlations

Family heritabilities ranged from  $0.61\pm0.12$  in HT20 to  $0.91\pm011$  in BB20 and  $0.98\pm0.11$  in Surv184. Height increment in 2020 and diameter growth in 2020 had moderate family heritability estimates (Table 4.5)

Generally, estimated genetic and phenotypic correlations in the study were in the same direction in sign and close in magnitude. Thus, here only the genetic correlations between the traits will be reported. The related tables could be consulted for further information about the phenotypic correlations (Table 4.6).

The genetic correlations between HT20 and Diam20 were strong and positive  $(0.85\pm0.06)$ . The families with high height increments also had high diameter growth, which was not surprising. However, the genetic correlations between bud set dates and growth traits were moderately high and adverse. Families with late bud set dates had less height increments and diameter growth than those with early bud sets. Interestingly, the families with early bud burst dates also had late bud settings (Table 4.6).

Traits	s w	Seed W	Seed T	Seed L	Germ %	Cot Num	Ht19	BB20	Surv 184	B S 2 0	Surv 364	Ht20	Diam20
SW	-	0.45 **	0.67 **	0.68 **	0.17*	0.38 **	0.31 **	0.0 6	0.1 5	-0.24**	0.02	0.61**	0.39**
SeedW		-	0.85	0.88 **	0.08	0.12	0.18 *	0.0 4	0.1 4	-0.11	0.04	0.27**	0.28**
SeedT			-	0.87 **	0.07	0.20 *	0.17 *	0.0 7	0.16*	-0.15	0.01	0.46**	0.30**
SeedL				-	0.09	0.18 *	0.21 *	- 0.0 2	0.0 4	-0.09	0.003	0.38**	0.30**
Germ (%)					-	0.22 *	- 0.17 *	- 0.1 0	- 0.0 5	0.002	0.12	0.18*	0.17*
Cot Num						-	0.24 *	0.1 5	0.20*	-0.17*	-0.01	0.49**	0.34**
Ht19							-	0.17*	0.36**	-0.23**	0.02	0.27**	0.25**
BB20								-	0.45**	-0.42**	-0.02	0.09	0.20*
Surv 184									-	-0.63**	0.27**	0.34**	0.47**
BS20										-	0.06	- 0.41**	- 0.51**
Surv 364											-	0.22*	0.23**
Ht20												-	0.64**
Diam20		· c'						1 1	** *				-

Table 4.3. Correlations between studied traits

ns - not significant; \* - significant at p < 0.05 level; \*\*- significant at p < 0.01 level

Table 4.4. Population means and standard deviation of means for germination, survival, and cotyledon numbers in phase I of the experiment (or in 2019).

Code	Average Germination Percentage (AGP)	Average Survival Rate(ASR)	Average Cotyledon Number (ACN)		
AKS	19.4±13.6	13.69±11.16	7.36±0.37		
UGU	36.6±13.46	29.14±13.61	7.5±0.29		
KIZ	35.26±14.9	26.4±11.9	7.26±0.14		
TAR	4.8±9.168	3.0±6.71	8.04±0.66		
FEK	34.1±15.05	42.03±14.31	7.34±0.22		
GÖK	30.7±15.96	26.37±11.9	7.3±0.20		
AND	11.65±16.22	8.5±	8.58±13.8		
DEV	30.76±19.9	24.7±18.2	7.40±0.28		

Table 4.5. *Mean squares, variance component as a percentage of the total variance (VC), and family heritabilities for the seedling traits* 

Traits	Replications	VC (%	Populations	VC (% of	Families/	V(% of to-	Error	VC(% of	Family
	df=5	of total varia- tion)	df=7	total var- iation)	Population df=141	tal varia- tion)C	df=5 59	total vari- ation)	heritability (h <sup>2</sup> f)
BB20	17.63 ns	0.9	585.80*	5.9	229.89**	63.6	20.5 7	29.5	0.91±0.11
Surv 184	0.00009 ns	4.6	0.0089 ns	1.1	0.01466**	85.1	0.00 028	9.2	0.98±0.11
Surv 364	0.00056 ns	0	0.0045 ns	0	0.0029**	19.4	0.00 14	80.6	0.52±0.12

Table 4.5 (Continued)

BS20	185.08 ns	0.1	1033.47**	2.2	224.05**	12.4	132.	85.3	0.41±0.12
							55		
Ht20	239.95 ns	0.9	6677.47**	41.5	204.69**	14.4	79.9	43.2	0.61±0.12
							3		
Diam20	12.38*	10.7	4.89**	0.3	1.199**	21.5	0.48	67.4	$0.60{\pm}0.12$
							5		

ns: not significant, \* significant at P < 0.05, \*\* significant at P < 0.

Table 4.6. *The correlations between the examined seedling qualities and their genetic (below diagonal) and phenotypic (above diagonal) components* 

Traits	BB20	SURV184	BS20	SURV364	HT20	DIAM20
BB20	-	$0.43 \pm 0.04$	$-0.29 \pm 0.05$	$-0.001 \pm 0.05$	$0.02 \pm 0.05$	0.14±0.05
SURV184	0.43±0.07	-	-0.47±0.04	0.25±0.05	0.22±0.05	0.29±0.05
BS20	-0.47±0.12	-0.77±0.10	-	0.05±0.06	-0.37±0.05	-0.41±0.05
SURV364	-0.002±0.12	0.34±0.10	0.10±0.18	-	0.18±0.06	0.16±0.06
HT20	0.03±0.11	0.28±0.10	-0.45±0.14	0.29±0.14	-	0.70±0.03
DIAM20	0.21±0.11	0.38±0.09	-0.62±0.14	0.27±0.104	0.85±0.06	-

# **CHAPTER 5**

#### DISCUSSION

#### 5.1. Nature of experiment and difficulties of establishing the experiment

The Cilician fir has unique niches in the Taurus Mountains in the Mediterranean region. Although the elevation range is between 1150 m and 2000 m, the optimum distribution is generally the Taurus Mountains' northern slopes and the elevational range of 1450-2000 m (Bozkuş 1988). Selection of study populations, cone collection, seed extraction, storing seeds, and optimizing germination procedures require adequate planning and labor-intensive work. More importantly, it is not easy to find a suitable forest nursery or test site where experience nursery experts could help to lay out the nursery tests and maintain them for several growing seasons. Considering these factors, the Olcay Nursery in the Konya seemed to be the right one to carry out the test. Despite all attention and care, the loss of experimental materials in 2019 and 2020 was considerably high due to high temperatures and drought. Nevertheless, the experiment with six replications produced reliable data with helpful information to understand how the species will cope with the changing environment in the future.

# 5.2. Seed and seedling traits relations with topography

Populations vary in terms of seed characteristics, particularly seed weight. Additionally, seed characteristics and topographical variables showed moderately strong negative associations. Higher latitude, elevations or altitudes in populations are typically associated with smaller seed weights and lower values of associated seed dimension features. It appears that seed weight and associated seed traits have adaptive significance. Those populations with more extended growing season requirements seem to have larger seed mass compared to those populations or families with shorter growing season lengths. The seed weight and associated traits may show clinal variation concerning topographical variables. However, these must be tested with the common garden beyond two growing seasons or long-term field tests. Similar findings were reported in studies of firs and other conifers in Turkey, including Anatolian black pine, Turkish red pine, and Scots pine in which found that the topographical factors might affect how populations differ genetically. (Işık, 1986; Kaya and Temerit, 1994; Kaya and Işık, 1997; Çiçek et al, 2005; Şevik et al., 2010).

#### 5.3 Seed traits and maternal effects

Seed weight and associated other seed traits varied significantly among populations. Families from low elevation and southern latitudes had larger seed weight than those families coming from northern latitudes and high elevations. Seed weight-related traits followed the same pattern. Between seed-related variables and growth traits, such as the quantity of cotyledons, height growths in 2019 and 2020, and diameter growth in 2020, there were relatively strong and favorable phenotypic relationships. These findings point out that Cilician fir has a strong maternal effect on seedling growth. This observation appeared to be valid for the first two years of seedling growth in the nursery environment. If early family evaluation is used for breeding, adaption, or assisted migration all only based on growth features; these results may mislead to identify good performing families in the future. In that instance, as seed effects are said to reduce with age (Kaya and Işık, 1997; Smith et al., 1993), such a selection technique does actually lead to changes in family ranking. The maternal influences on seedling growth will also have a significant impact on population selection based on early growth performances.

# 5.4. Genetic diversity patterns, family heritabilities, and genetic correlations between traits

Adaptive seedling features affected both the genetic diversity within and between populations. The population component of variance in growth traits is often small but important. The population component for height growth in 2020 was quite high.

On the other hand, for all seedling traits, the portion of overall variation attributable to families within populations was considerable. A similar result was found in the study by Gülcü et al. (2016). These findings imply that selection at the population and family levels will boost the genetic gain that breeding programs may be able to capture. However, the maternal effects caused by the seed weights on early seedling performances, especially growth traits, should be given special attention during the early selection of populations for adaptive plantations or families for breeding programs since the performance of populations and families could be stabilized in later years so the performance ranks of them may change. There are many examples of these performance rank differences in forest tree species if the selection of populations is based on seedling traits at an early age (Colbert et al., 1990)

Estimated family heritabilities for all seedling traits ranged from moderate to high, suggesting that selection based on family performances will be successful. Significantly, family heritability was high for the bud burst time. Although moderated to high family heritability estimates existed in seedling traits at the age of 2, it is expected that these estimated values will decrease in later ages after the maternal effects on growth traits are no longer effective on seedlings of families and populations (Smith et al., 1993; Kaya and Işık 1997).

In the growing season of 2020, there were moderately significant and adverse genetic connections between the period of bud set and height growth (-0.450.14) and diameter (-0.620.14). Interestingly, the genetic correlation between bud burst and bud set timing was moderately strong and negative (- $0.47\pm0.12$ ). The families with late bud set dates had less height and diameter growths. Moreover, those families with late bud burst dates had early bud set dates. These results indicated that those families with high growth performances had late bud burst and early bud set dates. In this way, seedlings could avoid early and late frost damage and efficiently use the growing season, meaning that seedlings complete their growth early in the summer when the water and heat stress is not yet high.

#### 5.5. Seed sources, climate change, and assisted migration

The study results provide crucial information on the magnitude and pattern of genetic diversity in seed and seedling traits. The population, as well as families within populations, significantly varied in these traits. In addition, a considerable portion of the total variation was heritable traits. The latitude, longitude and altitude seem to affect the seed and seedling traits. The variation in these traits may be clinal regarding the latitude, longitude and altitude gradients, but this has to be tested further. Significant maternal effects also caused varying seed weight on seedling growth performances in the first two growing seasons. In connection to the adaptational requirements of Cilician fir, the negative genetic correlations between phenological variables such as budset dates and growth traits appeared to be a key pattern.

From the above assessments of the results, the narrow natural distribution of Cilician fir in particular niches available in the Taurus mountains in the Mediterranean Geographic Region of Turkey forces populations and families with a short growing season length (late bud burst and early bud set) to avoid early and late frost damages. This short growing season is also favorable for avoiding water stresses which may develop later in Summer in high altitudes or higher latitudes. Assuming a climate change scenario of about a 2 °C temperature increase and a 20 percent decrease in precipitation (Önol and Semazzi, 2009), the species range is likely to expand up to 200 m upward in altitude and about 150 km north in latitude with less precipitation, the low elevation and low latitude population of Cilician fir will be helpful to genetic resources which could be used in the future. An assisted migration program in Cilician fir should be seriously considered by establishing tests beyond its natural range's altitudinal and latitudinal limits. Also, by evaluating seed stands and gene conservation forests established by the Forest Seed and Tree Breeding Research Directorate, new seed stands and gene conservation forests, especially populations from low elevation, latitudinal and longitudinal extremes of the species ranges, should be set up to be used in assisted migration experiments and plantations in the future.

# **CHAPTER 6**

#### CONCLUSION

In the current study, the pattern of genetic variation in adaptive seed and seedling traits of Cilician fir or Taurus fir (*Abies cilicica*) was studied using 8 populations representing the species' natural range for two years in a nursery experiment in the Olcay Forest Nursery in Konya.

Considering the natural habitats and growth requirements of the species in its natural environment, it was not easy to find a more suitable testing nursery than the Olcay Forest Nursery. Some precautionary measures were considered to reduce the effects of dry and hot weather. Despite these efforts, the germination and survival rates in the experiment were low. Nevertheless, the study generated invaluable genetic and ecological data, which could be used to mitigate the climate change impact on the species.

The findings of the study showed that adaptive seed and seedling features varied greatly among populations and families within populations. There may be a clinal variation with reference to altitude, longitude and latitude in these traits, according to the pattern of genetic diversity among populations. However, this must be further tested by establishing a common garden or field test beyond the two growing seasons. The variation due to populations varied from 0.3% in Diam20 to 41. 5 % in Ht20, while the variance component due to families within populations was from 12.2 % in BS20 to 85% in survival in the 2020 growing season. There was also moderate to high family heritability estimated for adaptive seedling traits. These results suggest that foresters will have opportunities to practice selection at the population and family within population levels.

Seed weight significantly and positively correlated with growth traits measured in 2019 and 2020, indicating the presence of maternal effects of seed weights on early performances of seedling's growth. In 2020, the amount and direction of the genetic and phenotypic associations between adaptive seedling qualities were the same. In the growing season of 2020, there were both strong and negative genetic correlations between bud set date and growth attributes.

It is interesting to note that during the same growth season, BB20 and BS20 showed both strong and negative genetic relationships. This indicates that the families who experienced late bud bursts had early bud sets and brief growing seasons.

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#### **APPENDICES**

# A. Analysis program sample data format used for Phase 1 experiment and Phase 2 experiments

Data collected from Phase 1 and 2 experiments were analyzed using the SAS statistical package (SAS Institute, 2018). Sample program scripts of SAS and data format of Phase 1 and 2 experiments were given below:

Phase 1 experiment

Sample program of SAS and part of the sample data

Scripts of SAS program to analyze the data of Phase 1 experiment

OPTIONS PS=60 LS=65 OBS=MAX;

LIBNAME murat 'c:\SASDATA';

DATA murat1;

INFILE 'c:\SASDATA\murat\MG12.txt' lrecl=9999 MISSOVER dlm='09'X dsd;

INPUT pop\$ fam lat lon alt sw1000 swidth sthick slength cot hy19 pger surv1 surv2 surv3 surv4 surv5;

RUN;

data m1;set murat1;

run;

data m2;set m1;run;

proc sort data=m2; by pop fam;

run;

proc print data=m2;run;

data m3; set m2;run;

proc sort data=m3; by pop; run;

proc means; by pop;

run;

data m4; set m3;

run;

proc corr data=m4;

var lat lon alt sw1000 swidth sthick slength cot hy19 pger surv1 surv2 surv3 surv4 surv5;

run;

data m5; set m4;

proc sort data=m5; by pop;

run;

••

••

The codes of data

Populations/Family/latitude/longitude/altitude/ seed weight/seed width seed thickness/cotyledon number/height growth 2019/percent of seed germination/ survival at date.. of/survival at date date.. of / survival at date date.. of/survival date date.. of /survival date date.. of.

TAR 1 37.09 34.62 1228 18.11 7.308 4.467 15.212 8 3.32 1.33 0.68 0.68 0.68 0.46 0.46 TAR 2 37.09 34.62 1242 18.99 7.885 4.504 15.468 8.75 3.09 2.6 7 1.39 2.43 2.43 1.68 1.68 37.09 34.62 1245 12.23 6.294 4.184 13.262 7 TAR 3 3.34 2.00 0.68 0.68 0.68 0.46 0.46 ••

37.07 31.82 1275 10.30 7.163 4.223 12.865 7.1 AKS 19 3.83 12. 00 7.06 6.67 6.67 4.44 4.17 20 37.07 31.82 1291 7.85 6.688 3.933 12.709 7.4 3.05 4.0 AKS 0 1.77 2.13 2.13 1.69 1.69 UGU 1 37.32 30.62 1152 19.83 6.998 4.894 14.519 8.1 3.54 53. 33 46.43 47.02 46.43 33.85 33.85 37.32 30.61 1180 23.14 7.760 4.818 15.440 7.5 UGU 2 6.23 54. 67 49.70 49.09 49.09 43.89 43.89 UGU 3 37.32 30.61 1184 19.56 7.806 4.841 15.675 7.5 5.29 36. 67 28.13 24.48 24.48 17.70 16.05 •• •• •• 38.34 36.07 1751 9.82 6.411 3.680 12.722 7.3 DEV 17 2.20 54. 00 46.67 45.45 45.45 30.30 30.30 DEV 18 38.34 36.07 1726 8.48 6.594 3.802 12.592 7.7 3.37 6.6 7 3.75 3.00 3.00 2.04 1.78 38.34 36.07 1714 10.62 6.522 3.935 12.728 7.3 DEV 19 2.33 36 .67 29.03 29.57 29.57 22.07 22.07 38.34 36.07 1721 7.47 0.000 0.000 0.000 7.8 2.44 7.3 DEV 20 3 3.75 3.75 3.37 2.04 2.04 Scripts of SAS program to analyze the data of Phase 2 experiment Part of the data from Phase I experiment: OPTIONS PS=60 LS=65 OBS=MAX: OPTIONS FORMCHAR="|---+=|-/<>\*";

LIBNAME bircan 'c:\SASDATA';

DATA murat1;

INFILE 'c:\SASDATA\murat\acmg8mart21.txt' lrecl=9999 MISSOVER dlm='09'X dsd;

INPUT rep pop fam sdl bb20 surv184 bs20 surv364 ht20 dm20;

RUN;

data m1;set murat1;

drop surv184 surv364;

gs20=bs20-bb20;

run;

data m2;set m1;run;

proc sort data=m2; by rep pop fam;

run;

proc means data=m2 noprint; by rep pop fam;

Var bb20 bs20 ht20 dm20 gs20;

output out=m23 mean=mbb20 mbs20 mht20 mdm20 mgs20 ;

run;

data m3;set m23;

run;

proc sort data=m3;by rep pop fam;

run;

data m4;set m3;

proc varcomp method=type1;

class rep pop fam;

model mbb20 mbs20 mht20 mdm20 mgs20=rep pop fam(pop);

run;

proc sort data=m4; by rep pop fam;

run;

data m42; set m4;run;

proc GLM;

Class rep pop fam;

model mbb20 mbs20 mht20 mdm20 mgs20=rep pop fam(pop);

manova h= rep pop fam(pop)/printh printe;

means pop/tukey duncan scheffe lsd;

run;

data m5; set m42;run;

proc sort data=m5; by pop; run;

proc means; by pop;

run;

data m6;set m5;

run;

proc corr data=m6;

var mbb20 mbs20 mht20 mdm20 mgs20;

run;

Part of the data from Phase two experiment:

The cods of data

Replication/Populations/Family/ seedling/budburst time (Julian days)/Julian date of survival-1/Bud set timing (Julian days) /date of survival-2/height growth 2020/diameter growth 2020 (Blanks are missing data, meaning seedlings were dead)

1	1	1	1	153	1	247	1	36.69 3.33
1	1	1	2	153	1	247	1	47.02 3.93
1	1	1	3					
1	1	1	4					
1	1	2	1	153	1	247	1	40.84 4.51
1	1	2	2	153	1	247	1	42.64 4.60
1	1	2	3	153	1	247	1	62.94 6.20
1	1	2	4	153	1	247	1	66.98 5.71
1	1	3	1	153	1	247	1	61.62 4.46
1	1	3	2	153	1	247	1	23.75 5.00
1	1	3	3	(	C	0	0.	00 0.00
1	1	3	4	(	0	0	0.	00 0.00
	2	21	1	153	1	247		
	2						1	
  1			2	153	1		1	53.69 5.90 40.82 2.99
  1	2	21	2 3	153 153	1	247 247	1 1 1	53.69 5.90 40.82 2.99
 1 1	2 2	21 21	2 3	153 153	1 1	247 247	1 1 1	<ul> <li>53.69 5.90</li> <li>40.82 2.99</li> <li>60.49 4.28</li> </ul>
 1 1	2 2	21 21	2 3	153 153	1 1	247 247	1 1 1	<ul> <li>53.69 5.90</li> <li>40.82 2.99</li> <li>60.49 4.28</li> </ul>
 1 1	2 2	21 21	2 3	153 153	1 1	247 247	1 1 1	<ul> <li>53.69 5.90</li> <li>40.82 2.99</li> <li>60.49 4.28</li> </ul>
 1 1	2 2	21 21	2 3	153 153	1 1	247 247	1 1 1	<ul> <li>53.69 5.90</li> <li>40.82 2.99</li> <li>60.49 4.28</li> </ul>

6	8	159	1	153	1	247	1	65.71	3.16
6	8	159	2	153	1	247	1	55.04	2.37
6	8	159	3	153	1	247	1	49.68	2.79
6	8	159	4	153	1	247	1	61.83	2.33
6	8	160	1						
6	8	160	2						
6	8	160	3						
6	8	160	4						

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Degree	Institution	Year of
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### PUBLICATIONS

1. Gülsoy, A.D., **Gülsoy, A. M.,** Çengel, B., and Kaya, Z. (2012). The evolutionary divergence of *Pinus nigra* subsp. *pallasiana* and its varieties based on noncoding trn regions of the chloroplast genome. Turk1sh Journal of Botany. 38. 627-636. 10.3906/bot-1312-26