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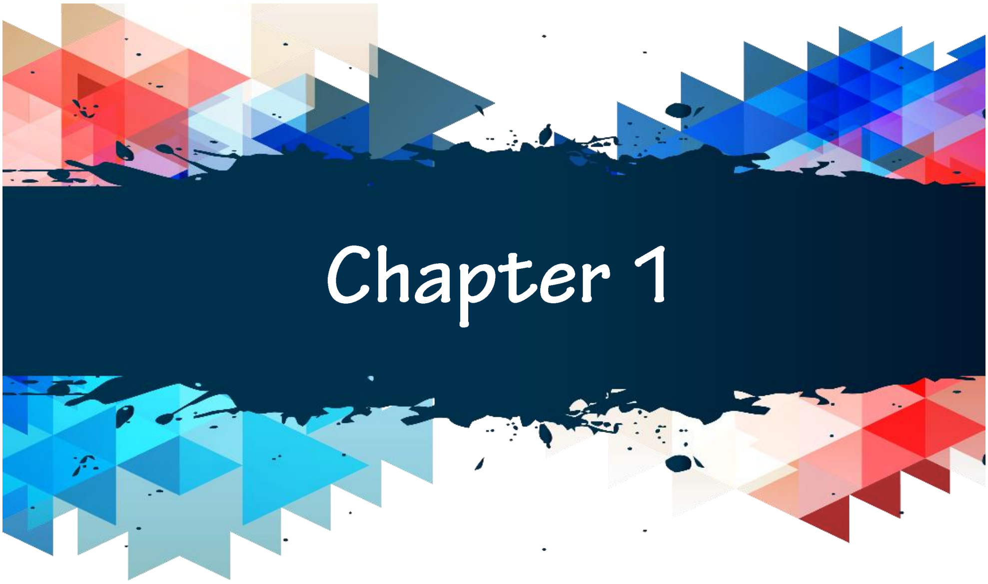

Chapter 1

The Role and Importance of the Western Rock Nuthatch (*Sitta neumayer* Michahellis, 1830) in Forest Ecosystems

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1. Introduction

Forest ecosystems have proven to be habitats of critical importance for the conservation of biodiversity and the maintenance of ecological processes. The health and sustainability of these ecosystems is directly related to the ecological functions of the species that comprise them. In this context, *Sitta neumayer* (Western Rock Nuthatch) has been identified as a key species that has a significant function in forest ecosystem processes. The aim of this study is to conduct a comprehensive investigation of the ecological role, habitat use patterns and conservation biology of *S. neumayer*.

1.1. Türkiye's Avian Biodiversity

Due to its location at the intersection of three different biogeographical regions (Euro-Siberian, Mediterranean and Iranian-Turanian), Türkiye has a great diversity of species. This unique geographical location makes the country an important center for bird species (Şekercioğlu et al., 2011). Current data show the presence of 496 bird species in Türkiye, which is about 75% of the total number of bird species in the European continent (Kuş Komitesi, 2024). Within Türkiye's bird diversity, resident species represent the largest group with a share of 33.3 %, followed by migratory birds (32.4 %), summer migratory birds (19.6 %) and winter migratory birds (14.7 %) (Kirwan et al., 2008).

1.2 The Sittidae Family and Its Species in Türkiye

The family Sittidae is a group of small to medium-sized (11-19 cm) passerines belonging to the order of passerines. The members of this family, which are represented by 29 species worldwide, are known for their compact body structure, strong beaks and characteristic climbing abilities. The most striking feature of these birds, whose backs are usually gray-blue in color, is their ability to climb upside down and move easily on flat surfaces. They are omnivorous and feed on insects and seeds. Members of the Sittidae live in monogamous pairs and build their nests in tree hollows or rock crevices, with most species closing the nest entrance with mud. These family members prefer forests, rocky areas and mountainous regions as their habitat and specialize in catching insects under tree bark and in rock crevices. They play important ecological roles such as pest control and seed dispersal within the ecosystem (Winkler, et al., 2020; Gill et al., 2024). The family Sittidae occupies an important place in the rich avifauna of Türkiye. According to the current state of research, there are four species of this family in our country, each with its own unique morphological and ecological characteristics. These species are *Sitta tephronota* (Eastern rock nuthatch), *Sitta europaea* (Eurasian nuthatch), *Sitta krueperi* (Anadolu's nuthatch) and *Sitta* *neumayer* (Western rock nuthatch) (Kiziroğlu, 2008; Trakus, 2024; Kuş Komitesi, 2024).).

1.3. Morphological and ecological characteristics of the species

Sitta tephronota Sharpe, 1872 (Eastern Rock Nuthatch) is the largest representative of the Sittidae family in Türkiye. Its body length varies between 16-18.5 cm and its body weight can reach 40-45 grams. Morphological diagnostic features include a gray-blue coloration of the dorsal region and a conspicuous black eye stripe. The ventral region is light beige-orange in color, and the bill structure is longer and stronger compared to other *Sitta* species (Cramp, 1993; Harrap & Quinn, 1996). In terms of habitat, the species prefers rocky hillsides, steep slopes and deep valleys and is generally observed in areas close to water sources. It prefers areas with dense vegetation of *Astragalus* spp., *Artemisia* spp. and species from the *Rosaceae* family. The species is often found in regions with *Prunus dulcis* and *Pistacia vera* trees and can also find habitats in sparse *Juniperus* and *Quercus* forests (Päckert et al., 2020; Harrap, 2020).

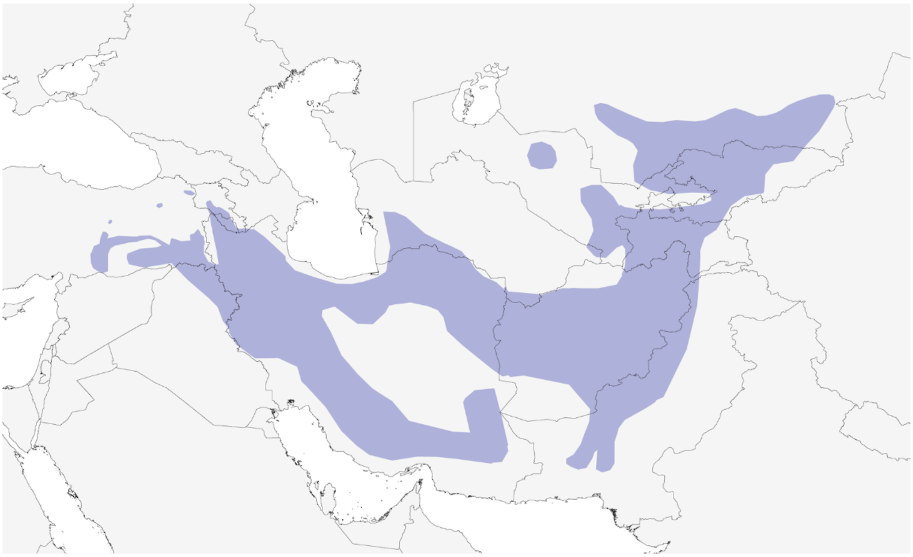

Figure 1: The worldwide distribution of *Sitta tephronota* (Birds of The World, 2024)

Sitta europaea L., 1758 (nuthatch) is a medium-sized member of the Sittidae family. Its body length varies between 13-14 cm. Morphologically, the back is blue-gray in color, while the belly is whitish and the cheeks are white. The body weight varies between 20-25 grams (Cramp, 1993; Harrap, 2020; Matthysen, 2010). As a habitat, the species prefers mature forest ecosystems with old and large trees, where a well-developed canopy is important to provide both feeding sites and the necessary cavities for nesting. The species is often observed in deciduous tree formations, especially *Quercus* species, and can also find habitats in forest ecosystems consisting of coniferous species such as *Picea* spp., *Pinus* spp., *Abies* spp., *Cedrus* spp. and *Larix* spp. (Winkler et al., 2020).

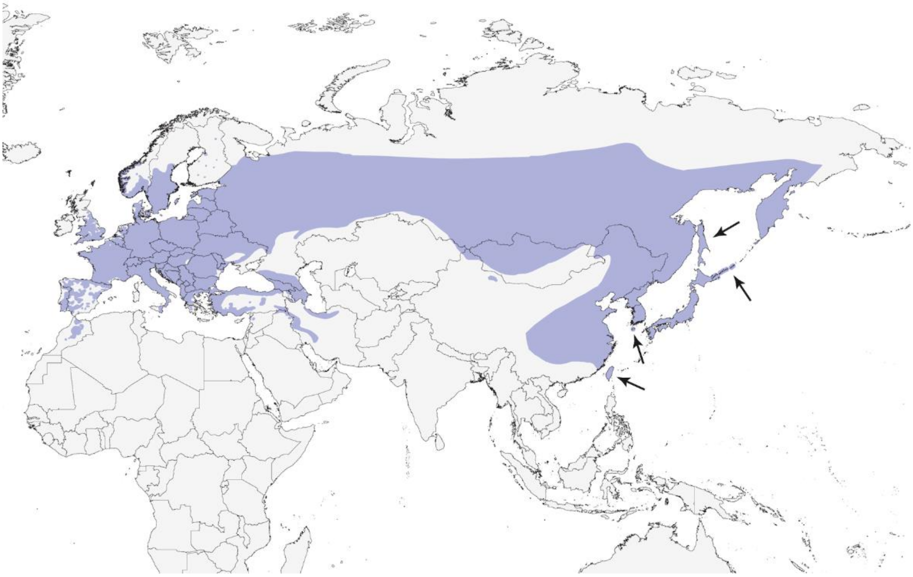

Figure 2. The worldwide distribution of *Sitta europaea* (Birds of The World, 2024)

Sitta krueperi Pelzeln, 1863 (Anadolu's Nuthatch) is one of the endemic bird species of Türkiye (Kiziroğlu, 2008). The diagnostic features of the species, whose body length varies between 11.5-12.5 cm, include a white-colored throat region, a black forehead and a chestnut-colored breast (Harrap & Quinn, 1996). The body weight varies between 15-20 grams (Matthysen, 2010). The species prefers mainly coniferous forests and is common in the mountainous regions of the Turkish Black Sea and Mediterranean regions. The species lives mainly in *Pinus brutia* forests, but is also observed in mountainous regions in formations of *Picea* spp., *Abies* spp., *Cedrus* spp. and *Juniperus* spp. The populations in the Caucasus prefer old *Abies* forests and mixed forest ecosystems with *Fagus* species. Although less common, the species also finds habitats in communities of *Acer* spp. and *Populus tremula* and mostly chooses conifers for its nesting activities (Harrap & Sharpe, 2020).

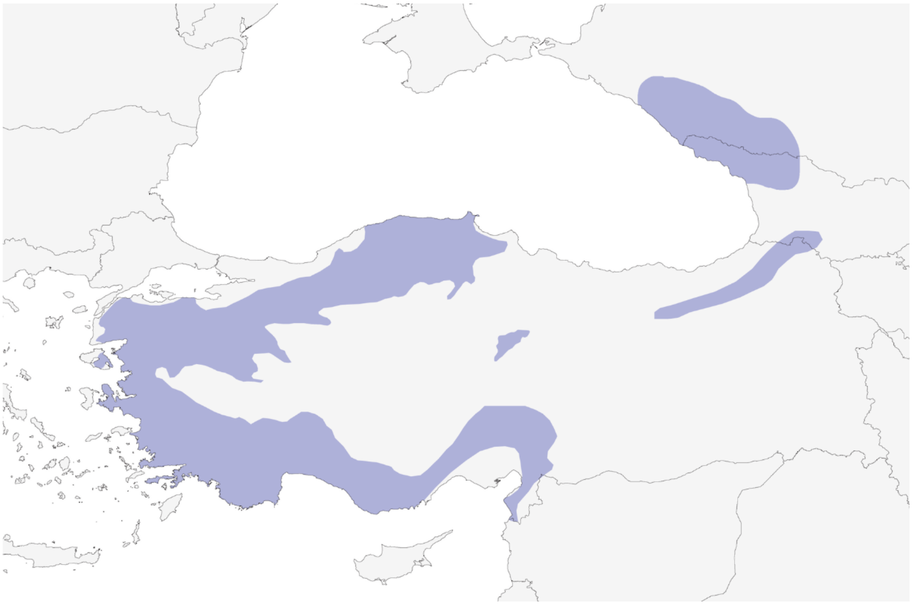

Figure 3: The worldwide distribution of Sitta krueperi (Birds of The World, 2024)

Sitta neumayer Michahellis, 1830 (Western Rock Nuthatch) is identified as a medium-sized species with a body length of 13.5-14.5 cm and a body weight of 20-30 grams (Harrap, 2020). Morphologically, the species, which has a gray-brown dorsal area, differs from the other species by a pronounced eye stripe and a white supercilium. The species, which has a long and robust bill structure, is known to prefer rocky areas for its nesting activities (Cramp & Perrins, 1993).

The morphological differences between these four Sitta species distributed in Türkiye probably reflect adaptations to their specific ecological niches (Matthysen, 2010). While *S. tephronota* and *S. neumayer* are specialized to rocky habitats, *S. europaea* and *S. krueperi* are adapted to forested areas (Harrap, 2020). This habitat specialization is reflected in the morphological characteristics of the species, such as bill morphology, body size and color patterns.

2. General characteristics and habitats of the Western Rock Nuthatch (*Sitta neumayer*)

Sitta neumayer Michahellis, 1830, is recognized as one of the medium-sized representatives of the family Sittidae. The body length of the species varies between 13-14.5 cm, the body weight is between 20-30 grams (Cramp, 1993). Diagnostic morphological features of the species include the gray-brown pigmentation of the dorsal region and wings with a characteristic black band running through the supercilium region. This characteristic band gives the species a masked appearance. The bill structure, which is an important adaptation for feeding strategies and nest building, is characterized by a long, robust and pointed shape (Figure 4).

Figure 4: Western Rock Nuthatch (*Sitta neumayer*) Safranbolu, Karabük

When investigating the habitat preference of *S. neumayer*, it was found that the species is mainly distributed in dry habitats such as rocky slopes, cliffs, passes and anthropogenic structures (old ruins, walls and historic buildings). The species is observed in high densities mainly in rocky maquis vegetation where limestone formations predominate (Roberge & Angelstam, 2006). It also occurs in barren areas and in areas with sparse herbaceous and shrub vegetation. It has also been found in forest ecosystems where scattered rock formations are present (Matthysen, 2010). The geographical distribution of the species is shown in Figure 5.

A modeling study conducted by Yousefi et al. (2020) documented the potential impact of climate change on the habitat suitability of *S. neumayer*. According to their results, the future range of the species could be significantly reduced due to rising temperatures and decreasing humidity.

Figure 5: Worldwide distribution of *Sitta neumayer* (Birds of The World, 2024)

2.1. feeding ecology of the western rock nuthatch (*Sitta neumayer*)

The feeding ecology and trophic interactions of *Sitta neumayer* are closely linked to the species' adaptations to its habitat. The species exhibits omnivorous feeding strategies and utilizes both animal and plant food resources. Detailed studies by Mohammadi et al. (2016) have shown that arthropods from the orders Coleoptera, Hemiptera and Lepidoptera play a dominant role in the diet of the species.

The food composition of the species shows considerable seasonal fluctuations. While arthropods make up 80-85% of the diet in spring and summer, this ratio drops to 50-55% in fall and winter, with the remaining portion of the diet being supplemented by seeds and fruits (Cramp, 1993). This trophic plasticity is considered an important life strategy that facilitates the adaptation of the species to variable environmental conditions.

Foraging strategies are basic behavioral adaptations that involve finding, acquiring and using food resources that organisms need to maintain their life functions. In this process, organisms develop strategies that maximize food acquisition while minimizing energy expenditure, according to the theory of optimal foraging. Factors such as the quality of food resources, their accessibility and the energy costs required to acquire them play an important role in determining these strategies.

S. neumayer occupies a position as a mesopredator in the trophic network of forest ecosystems and is of central importance for complex trophic interactions. According to calculations by Matthysen (2010), an individual regulates around 25,000-30,000 kilocalories of energy flow in the annual energy cycle and thus represents a significant component of energy transfer in the ecosystem (Sekercioglu, 2006). The metabolic analyzes of Cramp (1993) show that the species has a high energy assimilation efficiency, which plays a decisive role especially in meeting energy requirements during the breeding season.

The species acts as both predator and prey in the food chain. While it regulates arthropod populations as a primary consumer, it serves as potential prey for raptor species such as *Accipiter nisus*, *Falco naumanni* and *Strix aluco*. In terms of competitive relationships, it mainly competes with other insectivorous bird species for food resources. Competition with members of the Picidae family and other *Sitta* species is minimized by the different use of microhabitats and the diversity of foraging strategies (Roberge and Angelstam, 2006).

The trophic functions of species contribute significantly to the stability of the ecosystem. The mechanism of top-down regulation, particularly through the control of harmful arthropod populations, is critical to the health of forest ecosystems. The potential impact of climate change on arthropod phenology and the risk of disrupting food chain synchronization may necessitate the development of adaptive modifications to the feeding strategies of mesopredators such as *S. neumayer*.

Trophic interactions are fundamental mechanisms that regulate the flow of energy and the cycling of matter in ecosystems. These interactions, which manifest as direct interactions (predator-prey relationships), indirect interactions (competition, resource sharing) and interdependence relationships, are governed by top-down and bottom-up control mechanisms. Understanding this complex network of interactions is fundamental to the management and maintenance of ecological systems.

The function of seed dispersal of *Sitta neumayer* is considered a complex ecological process that contributes to the dynamic structure of forest ecosystems. The mechanisms of seed dispersal occur via two primary methods: endozoochory and synzoochory. In endozoochory, the small fruit-bearing seeds of species such as *Juniperus* spp., *Pistacia terebinthus*, *Crataegus* spp. and *Rosa canina* are transported through the digestive system to different habitats. The scarification effect of digestive enzymes on seed tegument results in a significant increase in germination success (Levey et al., 2005).

The synzoochory mechanism plays an effective role in the dispersal of seeds of species such as *Quercus* spp., *Pinus* spp. and *Fagus* spp. It has been observed that *S. neumayer* transports and stores these seeds over distances of 50-300 meters. The forgetting of about 25-30% of the stored seeds and the successful germination of some of these seeds help to maintain gene flow between populations, especially under fragmented habitat conditions.

The seasonal pattern of dispersal dynamics exhibits a structure that is synchronized with plant phenology. The phenological analyzes of Matthysen (2010) have shown that seed transport activity peaks in the period from September to November. This period coincides with the seed maturation of most woody plant species and provides optimal conditions for dispersal.

2.2 Reproductive biology and nesting characteristics

The breeding phenology of *Sitta neumayer* extends over a period from late March to mid-April. The species prefers to nest in rocky habitats. Nests built on lithological substrates show high success rates in rearing and survival of the young.

Nest-building behavior is one of the species' characteristic ethological adaptations. The nests are usually located in cracks and cavities in lithological substrates and are built from mud material. The modification of the nest entrance forms a protective barrier against predators. The average clutch size varies between 5-6 eggs and the incubation period is 15-17 days. The young leave the nest after a development period of about 20-22 days (Harrap, 2008; Shafaeipour et al., 2022).

Figure 6 shows the characteristic nest structure of *S. neumayer*, which is built on lithologic substrate. The species modifies the nest opening with mud, and this structural adaptation provides a protective function against predator pressure. The mud construction surrounding the edge of the crack or burrow in the lithologic substrate reflects the specific nidification architecture of the species. This structure also contributes to the maintenance of optimal microclimatic conditions for the development of the young. The documented nest morphology represents an important component of the species' adaptive behavioral repertoire, which is crucial for reproductive success. A study by Shafaeipour et al. (2020) has shown that the behavior of *S. neumayer* to contract the mud at the nest entrances not only serves as protection against predators, but is also an important mechanism for regulating the microclimate in the nest cavity.

Figure 6: A typical nest built by the Western rock nuthatch (*Sitta neumayer*) on a rocky surface. Safranbolu, Karabük

2.3. Ecosystem Services and Impacts on Biodiversity

Sitta neumayer has diverse ecological functions in forest ecosystems. The species is characterized above all as an effective biological control agent in regulating populations of insect pests. It has been found that a pair of *S. neumayer* consumes an average of 300-400 insects per day during the breeding season, reaching about 9,000-12,000 individuals per month. This predation pressure is an important mechanism for controlling pest populations, especially in the early stages of development.

The role of *S. neumayer* in seed dispersal is important for the survival of forest ecosystems. The species, whose diet consists mainly of fruits and seeds, plays an important role in the dispersal of seeds of species such as Juniperus spp, Pistacia terebinthus and Crataegus spp, especially in fall and winter (Levey et al., 2005).

It spreads the seeds both through the digestive system (endozoochory) and by dispersal (synzoochory) to different areas, thus contributing to greater plant diversity.

The presence of *S. neumayer* also has indirect effects on the diversity of other species. Species at the level of intermediate predators are considered important indicators of ecosystem health. The presence of this species in a habitat should be considered an indicator of healthy ecosystem functioning and high biodiversity potential (Roberge & Angelstam, 2006). This situation suggests that *S. neumayer* has characteristics of an "umbrella species" and can be considered a priority target species for conservation efforts.

The contribution of this species to genetic diversity is an important component of its ecological role. The large distribution range and seed production of *S. neumayer* promote genetic diversity by increasing gene flow between plant populations. This process is particularly important for improving the adaptability of plant populations in the face of environmental changes such as climate change. The role of this species in regulating insect diversity is also remarkable. Its regulating effect on insect populations helps to maintain insect diversity by controlling overly dominant species. This balancing mechanism is fundamental to the health and sustainability of forest ecosystems.

2.4 Threats and conservation strategies

Climate change is proving to be a significant threat to *S. neumayer* populations. It is assumed that every 1°C rise in average temperature can lead to an 8-10% decline in the breeding success of the species. This effect is particularly pronounced during the breeding season, as high temperatures can affect embryonic development and the survival rate of chicks. Studies have shown that the optimal temperature range for reproduction of this species is 18-28°C, with reproductive success decreasing significantly outside these limits (Yousefi et al., 2020).

Habitat fragmentation and loss also affect the genetic structure of populations. Studies on genetic diversity show that the heterozygosity rate in isolated populations is 15-20% lower. This situation threatens the adaptability and long-term viability of populations. Restricting gene flow between fragmented habitats exposes local populations to the risk of genetic drift and inbreeding depression (Nazarizadeh et al., 2016).

Habitat destruction by humans poses a significant threat to populations of *S. neumayer*. As Hadjibiros & Sifakaki (2009) emphasize, this situation should be made one of the priority targets of conservation strategies in Türkiye.

The dynamics of *S. neumayer* populations are crucial for the long-term survival of the species. Population density is directly related to the quality of the habitat. Factors such as the presence of rocky areas, vegetation structure and the availability of food resources play an important role in determining habitat quality. In particular, it has been found that breeding success correlates strongly with habitat quality (Matthysen, 2010).

When developing conservation strategies, the ecological requirements of the species must be considered in a holistic approach. The legal protection of priority habitats and the restoration of degraded habitats are of fundamental importance. The protection of rocky areas and forest-open land transition zones should be prioritized with regard to the habitat requirements of the species. Designing microhabitats that mitigate the effects of climate change can increase the species' tolerance to temperature stress. Temperature protection of nesting sites and sustainability of food resources should be among the primary objectives of adaptive management. Regular monitoring programs are essential for early detection of population trends and threat factors. Regular monitoring of genetic diversity should be used as an important tool to assess the long-term viability of populations.

2.5 Ecological characteristics and forest ecosystem associations of the Western Rock Nuthatch

Although the nominal name of *Sitta neumayer* refers to lithological habitats, recent research has described the species' interactions with forest ecosystems in more detail. The species is very frequently observed in ecotones between forest and lithological areas. These transition zones allow the species to make optimum use of the resources of both habitat types.

One of the most important functions of the species in forest ecosystems is its position in the trophic web. *S. neumayer* plays a crucial role in regulating the populations of wood-dwelling arthropods. In particular, arthropods inhabiting cavities and cracks in tree substrates are the main component of this species' diet, and this situation contributes directly to the health of the forest ecosystem.

Their physiological adaptations include complex mechanisms that ensure survival in different habitats. Their ability to thermoregulate has evolved as an effective adaptive response, especially against extreme thermal fluctuations in lithological habitats.

The ecological interactions of *S. neumayer* reflect a complex network structure that determines the position of the species within the ecosystem. Looking at the intraspecific interactions, a distinct territorial system can be observed. The territorial system is a behavioral and ecological organizational model in which organisms appropriate and defend a specific territory and protect it against conspecifics and sometimes also against heterospecific individuals. This system ensures the security of the spatial and trophic resources that individuals need to maintain their vital functions under conditions of limited resources.

The basic principle of territorial systems is based on the concept of a specific area (territory) that individuals or groups appropriate and defend. This spatial unit contains resources that are necessary for vital activities such as foraging, reproduction, nesting and the rearing of young. The spatial dimensions and characteristics of the territory vary according to the ecological requirements of the species, the distribution of trophic resources, the habitat parameters and the population density.

3. Conclusıons

Sitta neumayer is an important component of Türkiye's biodiversity. The ecological functions of the species are of fundamental importance, especially with regard to the control of pest populations and seed dispersal mechanisms. However, anthropogenic threats such as climate change and habitat loss are jeopardizing the long-term viability of the species.

A better understanding of the ecological requirements of the species and the development of appropriate implementation measures are necessary for the success of conservation strategies. In this context, the protection of the species' habitats, the maintenance of habitat connectivity and the systematic monitoring of populations are priorities. In addition, raising awareness among local communities and decision-makers is crucial to the success of conservation efforts.

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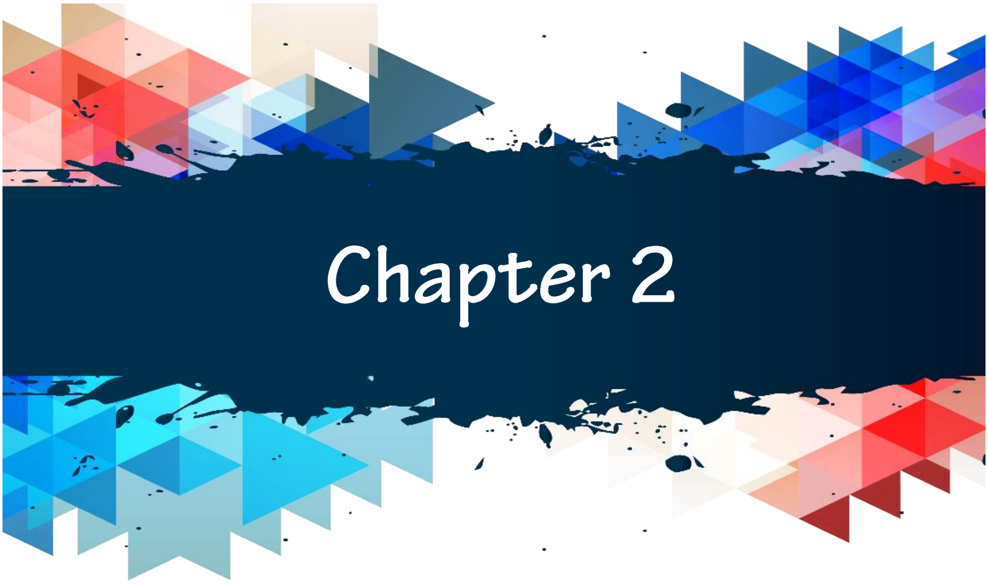

Chapter 2

The Morphological and Anatomical Variations in *Fagus orientalis* Lipsky Trees in Different Altitudes

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Introduction

Türkiye is among the countries that have attracted attention in recent years due to the increase in its forest resource since many developed countries have lost their forest resources day by day. Türkiye has a rich ecological diversity with an area of approximately 78 million hectares, and forest areas constitute an mean of 29.4% (22.9 million hectares) of the country's surface area (OGM, 2020).

In Türkiye, 32% of the forest area is covered by broad-leaved forests (tree species such as oak, beech, alder, chestnut, hornbeam), 48% by coniferous forests (tree species such as Red pine, black pine, Scots pine, fir, spruce, cedar), and 20% by coniferous + deciduous mixed forests (OGM, 2020).

In the forests of Türkiye, oak (*Quercus* spp.) (6.7 million ha) has the largest distribution area in forests, followed by Red pine (*Pinus brutia*) (5.2 million ha), black pine (*Pinus nigra*) (4.1 million ha), beech (*Fagus* spp.) (1.8 million ha), juniper (*Juniperus* spp.) (1.4 million ha), Scots pine (*Pinus sylvestris*) (1.4 million ha), fir (*Abies* spp.) (511,703 ha), cedar (*Cedrus* spp.) (402,319 ha), spruce (365,845 ha), stone pine (175,378 ha), alder (125,531 ha), chestnut (81,232 ha), hornbeam (55,654 ha), maritime pine (55,498 ha), poplar (22,525 ha), hazelnut (12,936 ha), laurel (12,184 ha) and ash (10,398 ha) in order of area size (OGM, 2020).

Trees are important living organisms in urban environments and forests (Özden, 2023; Özden, 2024) *Fagus orientalis* Lipsky is one of the most important forest trees of Türkiye and has both acological and economical importance (Şanlı, 1977). *F. orientalis* ranks fourth in Türkiye's current forest assets (OGM, 2020). *Fagus orientalis* forests cover an mean of 8.2% of total forest area of Türkiye with an area of 1,878,049 ha.

The beech tree (*Fagus* spp.), which is in the family Fagaceae, is represented by two species in Türkiye, namely the oriental beech (*Fagus orientalis* Lipsky.) and the European beech (*Fagus sylvatica* L.). However, only *F. orientalis* is naturally distributed in Türkiye (Atalay, 1992). In Türkiye, *F. orientalis* spreads from the Balkans in the west to Anatolia, the Caucasus, the northern part of the Elbruz Mountains and the Crimean Peninsula in the north of the Black Sea. In Türkiye, it has a fairly large distribution in Thrace, in the Istranca Mountains, Tekirdağ and Belgrade Forest, in the Aegean and Marmara basins, and in Northern Anatolia. It is also locally distributed in the Pos Forests of Adana in the south, in the north of the Amanos Mountains, in the Maraş-Andırın region (Saatçioğlu, 1969; Kayacık, 1980; Atalay, 1992; Yılmaz, 2009). Although its best development environment is generally between 700-800 m and 1100-1200 m altitudes, it can spread up to 1800-1900 m altitudes in the Eastern Black Sea Region (Atalay, 1992; Anşin and Özkan, 1997; Genç, 2004). *F. orientalis* is a first-class forest tree with a full and smooth trunk that can grow up to 30-40 m tall and over 1 m in diameter. The bark is light ash colored, thin and smooth (Anşin and Özkan, 1997). Young shoots are hairy and leaves are elliptical, pointed, long or short-tipped and 6-12 cm long. The lower surface of the leaves is hairy along the veins and the other parts are bare. There are 7-10 pairs of lateral veins. The leaf stalk is 5-15 mm long and hairy. *F. orientalis* is a shade-tolerant species; it is more widespread in shady aspects, mostly in the north and northwestern aspects.

Ecological factors are among the most important factors affecting growth and development in trees. Ecological factors affecting trees' growth and determining the nature of tree communities are divided into three groups: climatic factors (rain, wind, temperature, radiation, etc.), physiographic factors (altitude, aspect, slope, etc.) and edaphic factors. The survival of tree is affected by seasonal climate changes in the regions where they grow. Environmental factors such as accessibility to water, temperature, altitude, latitudes changing from south to north directly affect the anatomical properties of wood (Akkemik and Yaman, 2012). Wood anatomy is an important of science for many disciplines such as botany, ecology, forestry, forest industry, archaeology, paleobotany, architecture and has a long history. Understanding wood anatomy is of great importance in determining how tree species are affected by the environment in which they grow. The main factors affecting wood quality are the characteristics of the cells that make up the wood. Woods of the same species formed in different growing areas show different wood characteristics due to the effects of different factors such as geographical location and climate.

The altitude is one of the importants physiographic factors that most affect the anatomical characteristics of wood. In order for the *F. orientalis* tree to grow in the most suitable conditions, a certain decrease in temperature and an increase in precipitation are required. It is a species that prefers moist soils and has a high demand for air humidity. Being generally found on soils that are moderately moist and rich in mineral nutrients, it also leads to the development of live cover under loose shelter (Odabaşı et al., 2004; Kara, 2022; Kara and Özden Keleş, 2023). The most important factors in its growth are related to the altitude above sea level (Atalay, 1992). With the increase in altitude, temperature, relative humidity-water vapor ratio decreases and precipitation, evaporation and radiation intensity increase (Irmak, 1966; Atalay, 1983). Therefore, understanding the changes in the anatomical and morphological characteristics of the *F. orientalis* tree species, which is one of the most valuable tree species in Türkiye in terms of ecological and economic aspects, depending on the altitude and aspect will be decisive in increasing the sustainability of the *F. orientalis* trees in afforestation activities and determining the usage areas of its wood, and will benefit forestry activities.

Materials and Methods

In this study, *Fagus orientalis* Lipsky. (Oriental beech) tree species belonging to the Fagaceae family was selected. *F. orientalis* is economically and ecologically important in Turkish forestry. This study was conducted in Kastamonu which is located in northern of Türkiye. *F.orientalis* forests are mostly widespread in the coastal area of Kastamonu province. This study was thus carried out in *F. orientalis* forests located within the borders of different Operation Directorates affiliated to Kastamonu Forest Regional Directorate. The morphological and anatomical measurements were carried out in Daday and Cide regions of Kastamonu. Kastamonu has a continental climate with cold winters and rainy summers. The mean annual air temperature is 9.9 °C, ranging from −4.5 °C in January to 28.2 °C in August. The mean annual precipitation is 485.1 mm.

Figure 1. The study sites of *F. oriantalis* trees in Kastamonu (arrows show Daday and Cide regions)

The study areas were selected as being two different elevation steps (650 m and 1200 m). It was taken into consideration to have a difference of 500-600 m between both elevations, the lower elevation step in the study was formed by the *F. orientalis* forests located within the borders of Cide Forest Management Directorate and the higher elevation step was formed by the *F. orientalis* forests located within the borders of Daday Forest Management Directorate. Furthermore, morphological and anatomical measurements were made in two different aspects (north and south) in each elevation step.

Morphological measurements

The boundaries of 20x20 m sample areas of *F. orientalis* trees from Daday, Ballıdağ and Cide regions were determined, the trees entering the sample area were numbered clockwise and first the stem root diameters and then the diameters at breast height (1.3 m above the ground) of all trees in the trial area were measured from both directions using a caliper. At each elevation step, diameters at breast height and trunk length measurements were carried out on trees from the north and south aspects. Thus, morphological measurements were carried out on 50 trees at the low elevation, north aspect and 50 trees at the south aspect; at the upper elevation step, morphological measurements were carried out on 50 trees at the north aspect and 50 trees at the south aspect. Thus, a total of 200 trees were measured in total height and stem diameter. The diameters of the trees were determined using a caliper from breast height (1.3 m). The height of each *F. orientalis* tree from the ground was measured using a laser meter.

Anatomical measurements

To determine the age of the trees, transverse discs close to the root diameter were cut from both sampling areas. Then, separate discs were cut from the breast diameter. The cut transverse discs were placed in laboratory bags, then the discs were brought to the laboratory and the cambial age measurements were made on the discs. In order to view the annual rings on the discs more clearly, the discs were sanded with sandpaper until the surface was seen in the best way.

For anatomical measurements, wood pieces with an mean size of 1.5 x 1.5 x 1.5 cm were prepared from the discs cut from the samping areas and transverse, tangential and radial sections were prepared from these pieces. To prepare the micro-sections, small wood pieces were boiled in distilled water until they sank to the bottom to soften and remove air from their tissues, and the boiled samples were kept in equal amounts of alcohol-glycerin-distilled water until sectioning (Gerçek, 1997; Merev, 1998). Thus, sections were taken from the wood samples that were made suitable for sectioning using a slide microtome. Sections were prepared in the transverse, longitudinal radial and longitudinal tangential directions with a thickness of approximately 15-20 microns. In order to make the sections taken into continuous preparations, they were first made transparent in sodium hypochlorite for 15-20 minutes and then washed with distilled water. Afterwards, the samples were neutralized with acetic acid for 1-2 minutes, then washed again with distilled water and finally stained with safranin dye. After the staining process, the “Franklin” maceration method (Glacial Acetic Acid-Hydrogen Peroxide Solution at 1:1 volume) was used to release the wood elements in order to measure the liter cell length and width, trachea lumen width and trachea wall thickness dimensions that were not measured in the tissue. For this method, the samples were cut into matchstick-sized pieces before maceration. These pieces were then heated with 1:1 volumes of acetic acid and hydrochloric acid in a closed bottle and the maceration process was initiated. After the chemical reaction was completed, the middle lamella that provided the connection between the cells melted and the wood elements were separated from each other by means of a mechanical mixer. The released wood elements were washed with distilled water, filtered with a filter paper and then rinsed with alcohol. After the filtration process was completed, the obtained wood elements were treated with glycerin in small beakers and stained with safranin dye, and then preparations were prepared for measurements. Determination of cell dimensions was made using a Leica DM750 brand light microscope and Leica Application Suite (LAS EZ) image analysis program. From anatomical cell measurements, fiber length and width, vessel cell length and width, vessel lumen width, vessel wall thickness, core beam height and width were calculated.

Results and Discussions

In this study, the morphological and anatomical traits of the *F. orientalis* trees changed in 2 different altitudes (650 m and 1200 m) and 2 different aspects (north and south). *F. orientalis* showed different morpho-anatomical characteristics in different altitudes.

Morphological Results

Morphological measurements were carried out in Daday Ballıdağ (high altitude) forests and Cide forests (low altitude) where *F. orientalis* trees grow naturally in Kastamonu, and measurements were made separately for both north and south aspects. The diameter at breast height of *F. orientalis* growing at low (Cide) altitude levels was determined as 28.2 cm on mean in north aspects and 32 cm on mean in south aspects. The diameter at breast height of *F. orientalis* growing at higher (Daday Ballıdağ) altitude levels was determined as 26.2 cm on mean in north aspects and 24 cm on mean in south aspects.

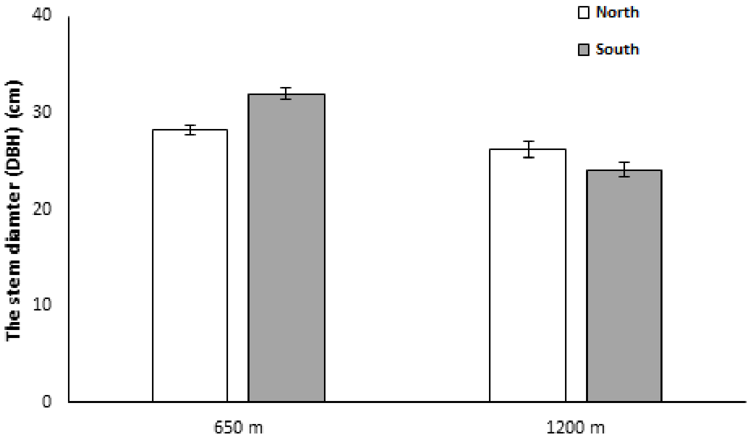

Figure 2. The mean stem diameters of F. orientalis between two altitudes (650 m vs. 1200 m) and aspects (North vs. South).

It can be seen in Fig. 3, the mean stem height of *F. orientalis* growing at low altitudes on the north aspect was determined as 24.4 m and the mean height value of *F. orientalis* growing at south aspect was determined as 24.5 m. The mean stem height of *F. orientalis* growing at high altitudes on the north aspect was determined as 20.8 m and the mean stem height of *F. orientalis* growing at south aspect was determined as 19.8 m.

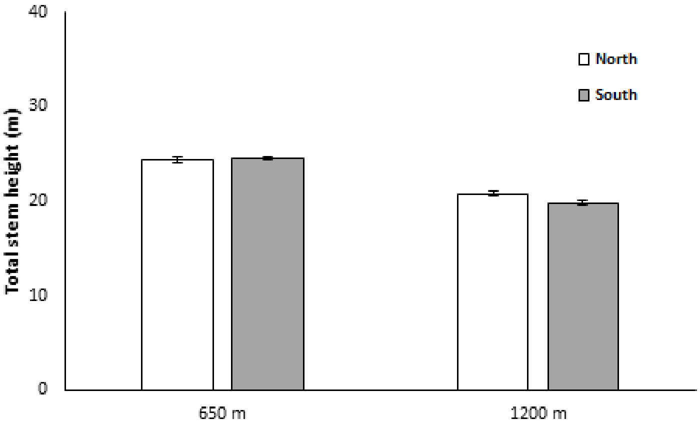

Figure 3. The mean total stem height of *F. orientalis* between two altitudes (650 m vs. 1200 m) and aspects (North vs. South).

Tree ring width and anatomical measurements

The annual ring measurements and anatomical cell measurement results indicated that *F. orientalis* growing in Daday Ballıdağ and Cide forests had significant differences in their tree ring width and anatomical traits. Tree ring widths were examined separately in both elevation steps and in each aspect. The mean annual ring width of the *F. orientalis* at 650 m elevation step was found as 1 mm in the north aspect and 0.62 mm in the south aspect. The mean tree ring width at 1200 m elevation step was determined as 1.1 mm in the north aspect and 0.77 mm in the south aspect. When the radial growth of the *F. orientalis* was examined, the tree rings were found to be wider in both elevation steps in the north aspects (Figure 4).

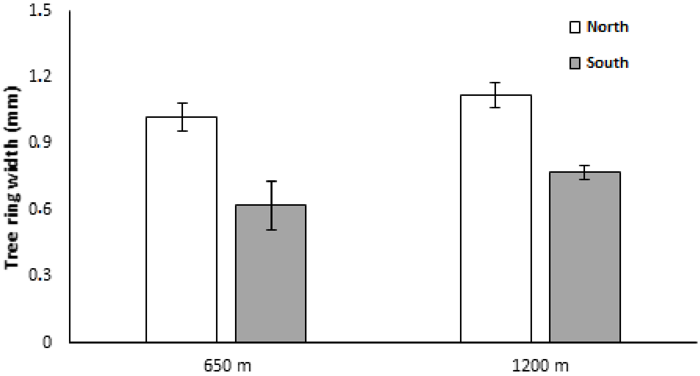

Figure 4. The mean tree ring widths of F. orientalis between two altitudes (650 m vs. 1200 m) and aspects (North vs. South).

Anatomical traits also differed between Daday and Cide. In Daday, the mean vessel diameter of *F. orientalis* trees growing in the northern aspect was determined as 78.9 µm, fiber lumen width (FLW) as 26.2 µm, mean fiber wall thickness (FWT) as 3.2 µm, mean ray height (RH) as 452.8 µm and mean ray width (RW) as 37.2 µm; in Daday, the mean vessel diameter (VD) of *F. orientalis* trees growing in the southern aspect was determined as 85.8 µm, FLW as 26.3 µm, mean FWT as 3 µm, mean RH as 443 µm and mean RW as 52.3 µm. The mean VD of *F. orientalis* trees growing in the northern aspect of Cide was determined as 41.5 µm, FLW as 45.1 µm, mean FWT as 6.4 µm, mean RH as 452.8 µm and mean RW as 37.2 µm; the mean VD of *F. orientalis* trees growing in the southern aspect of Cide was determined as 46.7 µm, FLW as 57.6 µm, mean FWT as 5.2 µm, mean RH as 400.3 µm and mean RW as 30.6 µm.

Table 1. The anatomical traits of F. orientalis between two altitudes (650 m vs. 1200 m) and aspects (North vs. South).

	Daday (1200 m)		**Cide (650 m)**	
Anatomical Traits	North	South	North	South
Vessel diameter (µm)	78.9	85.8	41.5	46.7
Fiber lumen width (mm)	26.2	26.3	45.1	57.6
Fiber wall thickness (mm)	3.2	3	6.4	5.2
Ray height (µm)	654.4	443	452.8	400.3
Ray width (µm)	77.5	52.3	37.2	30.6

Conclusion

F. orientalis is the most widespread tree species spreading in the temperate deciduous forests of the northern hemisphere. *F. orientalis,* which ranks second among deciduous trees in terms of the area it spreads in Türkiye. This study thus investigated how morphological, anatomical and tree ring widths of *F. orientalis* differed between two different altitudes (650 m and 1200 m) and two different aspects (north and south). The morphological characteristics showed significant differences between two altitudes. *F. orientalis* showed taller trees at 650 m altitude compared to 1200 m altitude. At an altitude of 650 m, The mean total stem heights of *F. orientalis* trees however did not show any difference between north and south aspects at an altitude of 650 m. The mean total stem height of the *F. orientalis* trees on the north side was found to be relatively taller than those on the south side at an altitude of 1200 m. The stem diameter of the *F. orientalis* trees was determined to be thicker at the 650 m elevation level than at the 1200 m elevation level. The stem diameters of the *F. orientalis* trees also showed significant differences between north and south sides. The tree ring widths varied significantly between two altitudes. *F. orientalis* trees showed wider tree rings at 1200 m altitude than at 650 m altitude. The tree ring widths of *F. orientalis* were found to be wider in the northern aspects compared to the southern aspects.

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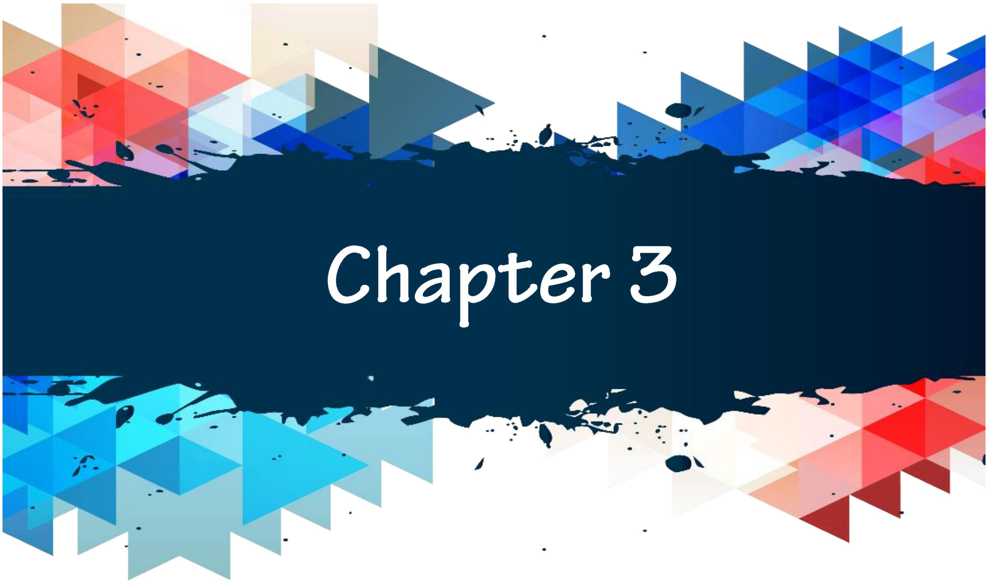

Chapter 3

Some Physiological and Biochemical Parameters Used in Drought Stress Estimation in Coniferous Tree Species

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1. Introduction

Climate change is increasingly disrupting the functionality and sustainability of ecosystems across the globe. Specifically, rising temperatures and altered precipitation patterns have made drought stress a prevalent challenge in forest ecosystems. This can lead to a reduction in the growth, biomass production and carbon sequestration capacities of forests, one of the main components of the carbon cycle. Drought is a climatic phenomenon characterized by prolonged periods of low rainfall when natural replenishment of water resources is insufficient. Drought is thought to have significant consequences on ecosystems and human life by creating environmental, economic and social impacts in both the short and long term.

Drought stress is the most significant environmental factor impacting crop yield (Bray, 1997), and the frequency of severe drought conditions is increasing due to global climate change (Dai, 2012). Plants have developed various morphological, physiological, and biochemical adaptations to survive drought stress in natural environments, as demonstrated by the vast diversity of plant species thriving in different climatic zones, including those with extremely arid conditions (Bohnert et al., 1995). The term “drought resistance” is more broadly used to describe plant species with adaptive traits that enable them to evade, avoid, or tolerate drought stress (Jones vd., 1981). The ability of a plant species to finish its life cycle prior to the beginning of drought is known as “drought escape”.

Coniferous tree species are an important component of forest ecosystems over a wide geographical area and are of great importance due to their economic, environmental and biological values. To date, many researchers have conducted research in many areas such as the identification and distribution of coniferous species, remote sensing monitoring, and products obtained from coniferous species (Bayram et al., 2023, 2024; Kaytanlıoğlu et al., 2023; Koparan, 2023; Alkan and Koparan, 2023; Nacakcı and Dutkuner, 2018; Özçelik et al., 2023; Özkan et al., 2023). Drought stress due to climate change threatens the growth dynamics, water use efficiency and survival of these species. In this context, accurate prediction of drought stress is a critical need for sustainable management of forests and adaptation to climate change.

Abiotic and biotic stresses are very effective on the development of plants. Drought is the most affected abiotic stress factor (Farooq et al., 2009). Drought stress triggers physiological, biochemical, and molecular responses in plants, enabling them to adapt to challenging growth conditions with limited resources (Hasegawa et al., 2000; Arora et al., 2002). Extreme drought and high temperatures lead to various morphological, physiological, biochemical, and molecular changes in plants. Moreover, drought is a critical stress factor in crop production, as it impacts nearly all plant functions and significantly reduces yield (Hernández et al., 2001). It also increases reactive oxygen compounds (ROS) that damage photosynthetic pigments, membrane lipids and proteins and affects enzyme systems (Yordanov et al., 2000; Egert and Tevini, 2002; Mittler 2006).

Many physiological traits that control the development and yield level of plants are also highly affected by drought. Half of the fresh weight of trees and about 89-90% of other plants is composed of water (Anjum et al., 2011). It is known that drought conditions at the time of seed formation cause 95% reduction in yield and water shortage, especially at flowering time, affects the reproductive status of the plant and causes infertility (Farooq et al., 2009).

In recent years, various predictive methods have been developed to evaluate drought stress in coniferous trees. These methods include physiological measurements, remote sensing techniques, modeling approaches and genetic analysis. The advantages and limitations of each method offer different solutions for researchers and practitioners. Many researchers apply methods developed in previous years in physiological stress studies (Scholander et al., 1965; Arnon, 1949; Heath and Packer, 1968; Bates et al., 1973; Velikova et al., 2000; Perez-Harguindeguy et al., 2013). This study aims to comprehensively examine the parameters used for drought stress prediction in coniferous tree species.

2. Some types of stress seen in plants

Abiotic and biotic stressors are critical elements that significantly affect the life cycle and productivity of plants. Abiotic stresses are caused by environmental conditions such as drought, salinity, temperature, nutrient deficiency or excess, metal toxicity, light imbalances and air pollution. Drought in particular is one of the main factors limiting plant growth due to low rainfall and anthropogenic errors in water management. In addition, biotic stresses are caused by the action of living organisms such as pathogens, herbivores, parasites and competition. Cellular and physiological changes caused by both abiotic and biotic stresses negatively affect photosynthesis, growth and development of plants, leading to losses in agricultural production. Understanding and managing these stressors is critical for sustainable agriculture and ecosystem balance.

3. Physiological parameters

3.1. Photosynthetic Gas exchanges

Photosynthetic gas exchange measurements are usually made using the LI-COR 6400 XT (Portable Photosynthesis system). In each measured tree, parameters such as net photosynthetic rate (Pn), known as carbon dioxide (CO2) assimilation rate, stomatal conductance (gs), intercellular CO2 concentration (Ci), transpiration rate (E), leaf temperature dependent vapor pressure deficit (VpdL), intercellular CO2 concentration/environmental CO2 concentration (Ci/Ca) and water use efficiency (Pn/E) are measured on a branch in the same direction.

By reducing leaf area and photosynthetic rate per unit leaf area, drought stress lowers photosynthesis. Stomatal closure or metabolic impairment are the primary causes of decreased photosynthetic rate (Tezara et al., 1999). Reactive oxygen species (ROS) are produced when photosynthetic light reactions continue under drought stress with limited intercellular CO2 concentration. This leads to the accumulation of reduced photosynthetic electron transport components, which may lower molecular oxygen. ROS have the potential to seriously harm the photosynthetic system (Lawlor and Cornic, 2002).

The xanthophyll cycle, the water-water cycle, the separation of light-harvesting complexes from photosynthetic reaction centers, and the thermal dissipation of excess light energy are among the adaptive strategies plants have developed to reduce drought-induced damage to photosynthesis (Niyogi, 1999; Demmig-Adams and Adams, 1996, 2006). Alterations in photosynthetic carbon metabolism are the main cause of metabolic disruption during drought stress (Lawlor and Cornic, 2002).

Under drought stress conditions, the most significant biochemical changes include the production of reactive oxygen species, such as singlet oxygen, superoxide anion, and hydrogen peroxide, accompanied by a decline in the photosynthesis rate (Bhargava and Sawant, 2013). Reactive oxygen compounds formed under stress are involved in cell metabolism and signal transduction mechanism (Cabello et al., 2014; Anjum et al., 2011). Too much accumulation of reactive oxygen compounds can cause cell death by causing lipid peroxidation and DNA fragmentation and therefore, the accumulation of these compounds should be prevented in drought conditions in order for plants to be resistant to stress conditions (Öztürk, 2015).

The parts of plants involved in photosynthesis are severely damaged even before the symptoms of extreme heat and drought (Crafts-Brandner and Salvucci, 2000; Camejo et al., 2005). Drought negatively affects the growth and development of cells by shrinking the stomatal apertures in the leaves of the plant. Water deficit leads to structural and functional changes in chloroplasts, resulting in a reduced rate of photosynthesis (Dubey, 1996). Water scarcity can cause photosynthesis to come to a standstill, affecting carbon assimilation and metabolism of the plant, leading to the cessation of life functions (Deltoro et al., 1998). When stomata close, carbon dioxide uptake is halted, leading to a decline in the net photosynthetic rate (Dubey, 1996; Deltoro et al., 1998; Farquhar and Sharkey, 1982). During water scarcity, reduced soil moisture lowers the water potential, and the subsequent decline in stomatal conductance leads to a reduction in the net photosynthetic rate (Epron and Dreyer, 1993; Lawlor and Cornic, 2002; Michelozzi et al., 2008). The season or time of day at which photosynthesis measurements are made also makes a significant difference in the amount of photosynthesis (Yang et al., 2002, Goudiaby et al., 2011). Some studies have also observed that high temperatures up to 40°C increase carbon fixation (Griffin et al., 2004).

In order to decrease transpiration under drought stress, plants that grow in arid environments have evolved xeromorphic characteristics. Leaf shedding (i.e., deciduous species under drought) and a reduction in the quantity, size, and branching of leaves are other ways that drought stress can reduce transpiration. Sclerophylly is another adaptive response to drought stress, where plants develop rigid leaves that can fully recover their functionality when normal conditions return, avoiding permanent damage from wilting (Micco and Aronne, 2002). Recent studies indicate that reduced stomatal conductance in response to drought stress is associated with structural features that decrease the chloroplast surface area exposed to intercellular spaces per unit leaf area, as well as reduced expression of aquaporin genes (Miyazawa et al., 2008; Tosens et al., 2012). Drought is known to interact with a number of other parameters, including as leaf developmental stage and light availability, to modulate mesophyll and chloroplast differentiation, which in turn affects conductance and photosynthetic capacity (Tosens et al., 2012).. Another adaptation for surviving in dry conditions is a decrease in the size and quantity of stomata. According to earlier research, stomatal density increases during mild drought stress but decreases during severe drought (Xu and Zhou, 2008). Therefore, all of these plant adaptations lessen the detrimental effects of drought stress on photosynthesis, which in turn improves water use efficiency (WUE) and raises yield potential and yield (Blum, 2005).

In case of extreme drought, the stomata in the leaves of plants shrink and the growth and development of cells slows down. Stress due to water deficit causes various problems in the chloroplasts of plants and causes stomata to close, thus reducing the rate of photosynthesis (Farquhar and Sharkey, 1982; Dubey, 1996; Deltoro et al., 1998). When water stress occurs, the decrease in soil moisture reduces the water potential, which in turn slows down stomatal conductance and photosynthetic rate (Epron and Dreyer, 1993; Lawlor and Cornic, 2002; Michelozzi et al., 2008; Xiang et al., 2013; Priwitzer et al., 2014). Due to water stress, it has been observed that there is a shrinkage in stomata and a decrease in CO_2 uptake, photosynthesis rate and respiration (Öpik and Rofle, 2005). It has been determined that in hot and arid environments, plants close their stomata and consequently, stomatal conductance and transpiration rate decrease and even defoliation occurs, resulting in a decrease in photosynthetic rate (Gratani et al., 2000; Li et al., 2004; Reddy et al., 2004; Yin et al., 2005; Guo et al., 2006; Wikberg and Ogren, 2007; Medrano et al., 2015; Ma et al., 2014; Pliura et al., 2018). The reduction in water use efficiency has also been attributed to a decline in stomatal conductance and an increase in the transpiration rate caused by high temperatures (Zhang et al., 2005; Kellomäki and Wang, 2001). Reduced water use efficiency has been observed in many plant species under water deficit conditions in hot and dry environments (Liang et al., 2006; Klein et al., 2001; Pliura et al., 2018). In arid conditions, both the photosynthesis rate and transpiration rate have been found to decrease, leading to a reduction in water use efficiency, which is the ratio of these two processes (Sinclair et al., 1984). In photosynthesis measurements, it is known that the photosynthetic rate varies according to the time of day when the measurements are performed (Goudiaby et al., 2011; Yang et al., 2002).

Stomatal conductance is influenced by various environmental factors, with vapor pressure deficit, soil water content, and soil and air temperature being the most significant (Miller and Schultz, 1987; Schäfer et al., 2000). Leaf temperature plays an active role on the vapor pressure deficit and, since air temperature varies during the day, it also has an effect on the water potential in leaves, stomatal conductance and transpiration (Conard et al., 1997). Stomatal closure significantly impacts both transpiration and photosynthesis (Medeiros et al., 2013; Júnior et al., 2019; Silva et al., 2013). In arid conditions, plants often close their stomata to minimize water loss through transpiration (Bosabalidis and Kofidis, 2002; Medeiros et al., 2013; Ribeiro et al., 2013), which reduces photosynthesis (Endres et al., 2010; Sales et al., 2012). Since the plant responds quickly to excessive water loss by preventing leaf dehydration, stomatal closure, although initially advantageous, becomes detrimental when prolonged as it interferes with CO2 flow (Machado et al., 2009; Inman-Bamber et al., 2012; Sales et al., 2012; Medeiros et al., 2013). In trees, when the crown of the tree is exposed to the sun, light and temperature increase and more vapor pressure deficit occurs, causing transpiration (Goudiaby et al., 2011). In addition, stomatal conductance and transpiration rate of the plant increase when the stomata are open (Goudiaby et al., 2011). In addition to the reduction in stomatal conductance, a decrease in intercellular CO_2 concentration has also been observed, provided the photosynthetic apparatus remains unaffected by water stress. (Kalaji et al., 2011; Medeiros et al., 2013; Ribeiro et al., 2013).

3.2. Chlorophyll, chlorophyll fluorescence and carotenoid pigment values

As chlorophyll pigment plays a crucial role in photosynthesis, it provides valuable insights into the metabolism, growth, and development of plants (Mencarelli and Saltveit, 1988; Bayçu and Özden, 2006). Carotenoids protect photosynthetic membranes from excessive amounts of light, play a role in the absorption and utilization of light for photosynthesis and are used to determine the physiological status of plants as much as chlorophyll (Eskling et al., 1997; Havaux, 1998; Yılmaz, 2013). Both chlorophyll and carotenoids are important indicators of the physiological status of plants (Yılmaz, 2013). The chlorophyll stability index is also a key indicator of a plant's stress tolerance capacity (Koleyoreas, 1958). Higher chlorophyll (Chl) and carotenoid (Car) contents have also been linked to the stress tolerance of plants (Sairam, 1994; Kraus et al., 1995). However, increased carotenoid content is also considered as protection against excess light (Lei et al., 2006). Several scientific studies have shown that drought can cause pigment degradation (Hendry et al., 1987), leading to irreversible damage to the photosynthetic apparatus due to water deficit (Clarke et al., 1996).

It is known that under adverse environmental conditions, such as seasonal water scarcity or abnormal light and temperature levels in summer and winter, chlorophyll synthesis is limited (Sauceda et al., 2008; Kancheva et al., 2014). It has also been determined by various researchers that plants experience a decrease in the amount of Chl a, Chl b, total chlorophyll and carotenoids under extreme conditions, especially under abnormal light and temperature conditions where water scarcity occurs (Yordanov et al., 2000; Ashraf, 2003; Pukacki and Kaminska-Rozek, 2005; Flexas et al., 2006; Sauceda et al., 2008; Terzi et al., 2010; Kancheva et al., 2014). In a study by Pilura et al. (2018), they determined that pigment values increased under hot and humid conditions and pigment production decreased under arid conditions in some species they studied. However, they found that both conditions (hot-humid and hot-arid) had a positive effect on pigment production in Yellow Pine, while the effect was always negative or neutral in European Spruce, another species studied.

Like extreme temperatures, intense light also causes a decrease in chlorophyll levels (Brett and Singer, 1973). Extreme drought disrupts the balance between the light absorbed by the leaves of plants and the light available to them, leading to a decrease in photosynthetic capacity (Foyer and Noctor, 2000). It has been found that leaves grown in sunny and shady areas differ morphologically, physiologically and biochemically (Nowak, 1991). Chlorophyll content is a biochemical property that can be easily affected by environmental conditions (Lepeduš et al., 2003). Plants grown in shaded environments have been observed to stabilize light absorption by increasing the pigment content per unit leaf area (Wittmann et al., 2001). In Sitka spruce, branches in shaded areas were found to have higher chlorophyll content (Lewandowska and Jarvis, 1977). In a study conducted in yellow pine, it was found that the amount of chlorophyll decreased from May to July (Kulaç, 2010). Chlorophyll pigment is also said to be affected by extreme light as well as extreme temperatures (Brett and Singer, 1973). In studies conducted according to aspect, it was reported that the amount of chlorophyll varies according to aspect (Nowak, 1991) and more chlorophyll was found in shaded aspects (Lewandowska and Jarvis, 1977; Wittmann et al., 2001). Gitelson et al. (1999) reported that the F735/F700 chlorophyll fluorescence ratio is positively correlated with the chlorophyll content in leaves, making this fluorescence ratio a useful tool for detecting chlorophyll levels. The CCM300 is a suitable instrument for measuring chlorophyll and chlorophyll fluorescence in the leaves of conifers and small plants that do not show a regular form (Repo et al., 2016; Ali et al., 2018; Marchetti et al., 2018).

3.3. Leaf characteristics

Since leaf area is related to the growth potential of plants, it is also important for determining the amount of photosynthesis. The photosynthetic ability of a plant is determined by the total leaf area and photosynthetic efficiency of its leaves (Çırak and Esendal, 2006). In addition, leaf area is used to determine volume in forests. There is an allometric relationship between leaf area and the diameter, height and volume of trees (Bozcuk, 1998; Kalıpsız, 1988; Carus and Çatal, 2005). Leaf dry weight (DW) is one of the methods used to detect stress in plants. In a study, it was determined that stressed plants were heavier in terms of dry weight than non-stressed plants (Garnier et al., 2001).

Specific leaf area (SLA) is a determinant for growth analyses and SLA is positively correlated with potential relative growth rate (RGR), photosynthetic rate and nitrogen (N) content in leaves, and higher SLA is associated with longer leaf life (Sadiklar, 2015). Non-nutrient-stressed species showed higher SLA values than stressed species and shade-tolerant species were also found to have higher SLA (Cornelissen et al., 2003). Leaf mass per leaf area (LMA) is used by many researchers in physiology studies as it is a marker of physiological characters such as carbon allocation, RGR, photosynthetic rate, and leaf longevity (Wright and Westoby, 2002; Poorter et al., 2009). In some studies, species with higher LMA values were found to have better average leaf longevity in various growing environments (Diemer, 1998; Ryser and Urbas, 2000). Leaf dry matter content (LDMC) provides information about leaf elasticity and stiffness and higher LDMC means that leaves have thicker and stiffer cell walls (Sadiklar, 2015). Stiffer and thicker leaves provide turgor at lower water potential and cells are less damaged under drought stress (Monson and Smith, 1982; Engelbrecht and Kursar 2003; Tyree et al., 2003).

Nowadays, leaf length values are mostly used for leaf area calculations (Flower-Ellis and Olsson, 1993). Leaf area is a critical growth parameter and must be measured to effectively monitor and assess plant growth and development (Kandiannan et al., 2009). Leaf area is also a key variable in agronomic and physiological studies, as it plays a vital role in processes such as light capture, photosynthetic efficiency, evaporation, and plant responses to fertilization and irrigation (Blanco and Folegatti, 2005). It is possible to obtain more accurate information about both leaf size and leaf area by developing a model utilizing leaf width values as well as leaf length in leaf area estimations (Williams and Martinson, 2003; Cho et al., 2007; Peksen, 2007; Antunes et al., 2008; Kandiannan, 2009).

Leaf thickness plays a crucial role in leaf and plant development and is closely linked to species' nutrient acquisition and utilization strategies. The amount of light absorbed by a leaf and the diffusion pathway of CO_2 through its tissues depend, at least partially, on leaf thickness (Agustí et al., 1994; Syvertsen et al., 1995). Under drought stress, plants can increase leaf thickness as a strategy to retain more water within their leaves (Lopez et al., 1997; Trujillo et al., 2013).

Specific leaf area (SLA), defined as the area of one side of a fresh leaf divided by its oven-dried mass, serves as an indicator of plant growth status (Sadıklar, 2015). A strong relationship has been observed between SLA and leaf thickness (Wilson et al., 1999). Plants grown in high-light environments typically develop thicker leaves with lower SLA values (Björkman, 1981). Furthermore, Cornelissen et al. (2003) reported that non-stressed plant species exhibit higher SLA compared to stressed species.

Plant traits are widely utilized to describe the functional aspects of plants and their interactions with environmental conditions (Verheijen et al., 2015). Notably, leaf mass per area (LMA) is central to a suite of traits influencing plant functional ecology (Shipley, 1995; Wright et al., 2004; Niinemets, 2015). LMA holds critical functional significance as a key trait in carbon fixation strategies (Wright and Westoby, 2002), scaling with leaf biomass investment per unit area and per unit mass (Niinemets, 1999, 2001; Gratani and Varone, 2006; Poorter et al., 2009; Puglielli et al., 2015). LMA responds to climatic variations through modifications in components such as leaf thickness (Lt) and leaf tissue density (Ltd) (Bertin and Gary, 1998; Gratani and Varone, 2006; Gratani et al., 2018).

Perennial plant species in arid and nutrient-poor growing environments are reported to have thicker and stiffer leaves (Chapin, 1980; Fonseca et al., 2000). The commonly used determinant in assessing leaf form is leaf mass per area (LMA), which is the product of leaf thickness and leaf dry matter content (LDMC) (Witkowski and Lamont, 1991). Species with low LMA generally exhibit a larger light capture area per unit mass, higher leaf nitrogen concentration, and greater photosynthetic capacity per unit leaf mass (Field and Mooney, 1986; Reich et al., 1997; Wright et al., 2001; Parkhurst, 1994). In species with high LMA found in arid habitats, common leaf traits such as thick, stiff leaves and small, thick-walled cells are often interpreted as adaptations to water stress (Maximov, 1929; Cunningham et al., 1999; Niinemets, 2001). Similarly, these leaf traits, along with high LDMC values, in species growing in nutrient-poor soils are considered adaptations to extend leaf lifespan in environments where rapid growth is not feasible (Grime, 1977; Grubb, 1986; Chapin et al., 1993; Turner, 1994).

The higher the LDMC value, the more durable, hard and thick cell walls the leaf has (Sadiklar, 2015). It is also known that LDMC is related to water potential (Kusar et al., 2009). Accordingly, LDMC data can be obtained to determine the degree to which plants can withstand drought (Markesteijn et al., 2011).

3.4. Leaf water potential

There are three primary reasons why leaf hydraulics might be important in this adjustment. First of all, the leaves are the most distant component along the soil-plant hydraulic system, the main site of exchange, and the organs that bear stomata. Therefore, the organs with the highest negative water potentials are the leaves, which are also ultimately in charge of regulating the water potential throughout the entire plant. According to the second vulnerability segmentation concept, leaves are more prone to hydraulic damage compared to other plant organs such as stems and branches (Charrier et al., 2016; Scoffoni and Sack, 2017; Tyree and Ewers, 1991; Zhu et al., 2016). Leaves may act as a "safety valve," protecting the rest of the plant from excessively negative water potentials, as they are expected to be the first organs to exhibit hydraulic failure during severe water stress (Scoffoni and Sack, 2017; Tyree et al., 1991; Wolfe et al., 2016). Furthermore, both xylem and outside-xylem pathways contribute to leaf hydraulic conductance and vulnerability to hydraulic failure, and these pathways may respond differently to water stress (Cochard et al., 2004; Scoffoni and Sack, 2017; Scoffoni et al., 2017; Trifilò et al., 2016). As a result, predicting the hydraulic responses of leaves to drought is more complex than for branches. However, leaves are also more likely to adapt and exhibit plastic responses to drought conditions (Martorell et al., 2015).

Leaf water potential affects stomatal conductance and transpiration rate (Conard et al., 1997; Endres et al., 2010; Silva et al., 2013). Many studies have found a negative correlation between decreased water potential (increased water stress) and proline (Lansac et al., 1994; Kandemir, 2002); Cai et al., 2007; Yang et al., 2007). Leaf water potential indicates the water tension within the leaf, which drives the movement of water from the plant to the atmosphere. It is widely utilized in studies examining the effects of water stress on plants (Bergonci et al., 2000). In a study conducted in larch, it was observed that water potential values decreased as water stress increased (Demir, 2019). Midday water potential is one of the traits measured by researchers as it is an indicator of water stress in plants (Naor, 2000; Intrigliolo and Castel, 2006).

3.5. Proline

Plants adapt their metabolism to environmental conditions by osmotic regulators such as proline, glycine and betaine under extreme seasonal conditions in their habitats (Türkan, 2008). The main function of proline is to protect cells from osmotic damage by providing osmotic adjustment and to protect plants against various environmental stresses by eliminating ROS species formed under stress (Szabados and Savouré, 2010; Roychoudhury et al., 2015). Proline accumulation occurs in extreme drought conditions and acts as a defense against membrane damage and tries to minimize the damage to enzymes (Iyer and Caplan, 1998; Kishor et al., 2005; Tanner, 2008; Szabados and Savouré, 2010; Yavaş et al., 2016; Moustakas et al., 2011; Singh et al., 2015). For these reasons, proline is used to determine the physiological status of plants (Kishor and Sreenivasulu, 2014; Yılmaz, 2013).

Some researchers have observed that the amount of proline increases during drought under water stress (Lansac et al., 1994; Caı et al., 2007; Yang et al., 2007; Kacar and Katkat, 1998; Öpik and Rofle, 2005; Shyaleva et al., 2005; Kulaç, 2010; Saruhan Güler et al., 2012; Terzi et al., 2015; Altuntaş et al., 2019). Akça and Yazıcı (1999) concluded that proline levels increased during dry times in summer in their study conducted in Sarıçam and Kızılçam in Mediterranean climate type. Kulaç (2010), in a study conducted on Scots pine, found that while the amount of proline was low in the April-May period due to water stress, the amount of proline in the needle leaves of the origins increased during dry periods. In a study conducted on various plant species, it was found that the amount of proline was higher in stress-resistant species than in sensitive species (Ashraf and Foolad, 2007). In addition, proline has been found to eliminate ROS in some studies (Szabados and Savouré, 2010; Roychoudhury et al., 2015). In studies conducted in different seasons in yellow pine and red pine, which is one of the main species of the region where the study area is located, higher proline content was observed in the spring season compared to the summer season, and a decrease of half in proline between seasons was detected in red pine, while no significant decrease was observed in yellow pine (Yılmaz, 2013). Näsholm and Ericsson (1990) observed that in Scots pine, proline levels were lower in the so-called early spring than in other periods. In an age-related study, pigment and proline were found to be higher in 18-year-old Scots pine needles than in 30-year-old Scots pine needles (Turfan et al., 2016). Altuntaş et al. (2020) also determined that proline application is an application that increases the amount of photosynthesis in the leaf, and photosynthesis increases if it is given to plants at low levels.

3.6. Hydrogen peroxide

Reactive oxygen species (ROS), including molecules such as H_2O_2, superoxide, hydroxyl radicals, and singlet oxygen, are found in cellular compartments like chloroplasts and mitochondria (Anjum et al., 2011; Cabello et al., 2014; Lee and Park, 2012; Bhargava and Sawant, 2013). ROS play a dual role in plants, functioning not only as harmful agents but also as signaling molecules involved in regulating growth, development, responses to biotic and abiotic stresses, and programmed cell death (Foyer et al., 1997; Bailey-Serres and Mittler, 2006). To mitigate ROS-induced damage, plants adjust their metabolism through various strategies, such as producing adaptive compounds, synthesizing regulatory proteins, and activating antioxidant defense mechanisms (Krasensky and Jonak, 2012).

Drought stress can increase ROS production in plants, causing excessive accumulation of ROS that can lead to lipid peroxidation, DNA damage and cell death (Cruz de Carvalho, 2008; Hossain et al., 2015; Öztürk, 2015). H_2O_2 has a central role in the responses of plants to both biotic and abiotic stresses (Demiralay et al., 2013). For instance, H_2O_2 has been shown to play a significant role in the adaptation of *Cistus albidus* to summer drought (Jubany-Marí et al., 2009), in maize under salt stress (Azevedo Neto et al., 2005), and in enhancing soluble sugar content in melon fruits (Ozaki et al., 2009). Furthermore, Yang et al. (2009) found that H_2O_2 promoted adaptation responses by triggering proline accumulation in maize seedlings, suggesting a positive relationship.

Altuntaş et al. (2019) suggested that H_2O_2 may increase drought tolerance by interacting with genes regulating proline and polyamine metabolisms. Studies on various plants have shown that exogenous H_2O_2 application enhances antioxidant activities, reduces lipid peroxidation, and improves the plants' ability to adapt to environmental stresses such as temperature, frost, and salinity (Azevedo Neto et al., 2005; Wahid et al., 2007). Additionally, Wahid et al. (2011) reported that H_2O_2 application delayed stomatal closure and mitigated the decline in the photosynthesis rate by maintaining leaf water content during drought stress.

3.7. Lipid peroxidation

Peroxidation causes damage to the lipids that make up cellular membranes during drought stress (Jiang and Huang, 2001). Compared to species that are more vulnerable to drought, plant species that are more tolerant of drought stress show higher levels of antioxidant activity (Turkan et al., 2005). Additionally, heat stress causes lipid membrane peroxidation and an increase in ROS generation (Xu et al., 2006; Liu and Huang, 2000). Compared to their less tolerant relatives, wheat (Triticum aestivum L.) (Sairam et al., 2000) and cotton (Gossypium hirsutum L.) (Sekmen et al., 2014) varieties with improved heat tolerance also show noticeably higher levels of antioxidant activity. In Kentucky bluegrass (Poa pratensis L.) and tall fescue (Festuca arundinacea Schreb), membrane lipid peroxidation increases more rapidly when drought and heat stresses are combined compared to each stress individually. This accelerated membrane damage is accompanied by reduced antioxidant activity, suggesting that the combined stress conditions overwhelm the defense mechanisms responsible for stabilizing plant membranes (Jiang and Huang, 2001). Crop responses to heat and drought are likely significantly influenced by the capacity of antioxidant systems to protect plant membranes and physiological processes (Sekmen et al., 2014). Analyzing the activity of key antioxidants, chlorophyll fluorescence (ChlF), and lipid peroxidation can help evaluate the functioning of photosynthetic thylakoid membranes and the defense mechanisms that maintain photosynthetic rate (PN) during severe oxidative stress.

Lipid peroxidation determination is a commonly used method to assess the extent of cellular damage in plants. The level of malondialdehyde (MDA), a byproduct of lipid peroxidation, is measured to evaluate oxidative damage (Smirnoff, 1995; Özden, 2009). Studies have reported increased MDA levels in water-stressed plants, whereas stress-resistant species exhibit lower lipid peroxidation (Pastori and Trippi, 1992; Sairam et al., 1997, 1998; Terzi and Kadıoğlu, 2006). Other researchers noted that higher MDA levels reduce drought resistance and increase with plant aging (Turfan et al., 2016). Prolonged drought stress has been associated with increased MDA accumulation, along with reduced relative water content and photosynthetic pigment levels (Jiang and Huang, 2001).

4. Conclusion

In this study, various physiological and biochemical parameters and methods for predicting drought stress responses in coniferous tree species were comprehensively examined. Increasing temperatures and irregular precipitation regimes caused by global climate change pose significant threats to forest ecosystems. Drought stress limits the growth, biomass production, and water-use efficiency of coniferous species, thereby reducing their survival capacity.

The primary physiological parameters addressed in this study include photosynthetic gas exchange, stomatal conductance, chlorophyll and carotenoid pigment values, leaf traits, leaf water potential, malondialdehyde accumulation, and proline accumulation. Changes in stomatal conductance and transpiration rate in photosynthetic processes have been found to be critical determinants in plant adaptations to drought stress. Additionally, the increase in osmoprotectants such as proline accumulation demonstrates that plants activate defense mechanisms under stressful conditions. Morphological traits, including leaf area, leaf thickness, and specific leaf area, were found to play an essential role in the adaptation of species to drought conditions. In this context, structural changes have been observed to limit water loss and influence photosynthetic capacity.

In conclusion, the parameters examined in this study contribute to understanding the adaptation mechanisms of coniferous tree species to drought stress and provide a foundation for developing predictive methods for their sustainable management. Multidisciplinary approaches are required to enhance the adaptive capacity of forest ecosystems to climate change and to maintain their functionality. Future studies incorporating new technologies such as genetic analysis and remote sensing could further advance this field.

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