

ANTIOXIDANT VARIABILITY OF THE SEEDS IN CORE AND MARGINAL POPULATIONS OF TAURUS CEDAR (*Cedrus libani* A. Rich.)

ANTIOKSIDACIJSKA VARIJABILNOST SJEMENA U GLAVNIM I MARGINALNIM POPULACIJAMA LIBANONOSKOG CEDRA (*Cedrus libani* A. Rich.)

Sezgin AYAN¹, Nezahat TURFAN², Esra Nurten YER¹, Muhidin ŠEHO³, Halil Barış ÖZEL⁴, Fulvio DUCCI⁵

Summary

Genetic diversity is the basis for adaptation and survival of tree species under changing environmental conditions, representing the key issue of stability and productivity of forest ecosystems. In this study, core and marginal populations of Taurus cedar (*Cedrus libani* A. Rich.) were investigated due to their importance in gene conservation. Assessment of genetic diversity in isolated populations is of great importance for the conservation and improvement programs. Under global climate change conditions, they may possess genotypes of future adaptive potential. The aim of this study is to determine the amount of proline to understand water deficiency stress of the population, total soluble proteins, MDA, H₂O₂, α-amylase, the variability of antioxidant as CAT, SOD, APX and GuPX of Taurus Cedar seeds from five core populations (Kahramanmaraş-Andırın/Elmadağı (AND), Adana-Pozantı/Pozantı (POZ), Mersin-Anamur/Abanoz (ANA), Antalya-Finike/Aykırıçay (FIN) and Antalya-Kaş/Karaçay (KAS)) and one marginal provenance (Amasya-Tokat-Niksar/Çatalan (NIK)) in Turkey. According to the results, a significant difference was detected between populations. Significantly higher amounts of proline were detected for ANA (7,46 µmol/g) and POZ (7,22 µmol/g) populations, whereas the lowest amounts of proline were detected in KAS (3,98 µmol/g) population, which represent the optimal distribution of Taurus cedar. This finding indicates that POZ and ANA populations, in the transition zone from Mediterranean region to steppe territory, are more resistant to the frost, than the other populations. The highest α-amylase enzyme amount was detected in POZ population, growing in the optimum range for Taurus cedar. Significantly higher levels of H₂O₂ were detected in NIK (11,97 µmol/g) and ANA (11,60 µmol/g). This is an indication of higher levels of oxidative stress in the seed samples of these populations. With the present research it's verified that, enzymes such as SOD, CAT, GuPX and APX, controlling reactive oxygen species (ROS) levels in plant cells, are the elements of the antioxidant defence system functioning as protective mechanisms for plants against stress conditions. From the practical point of view, improvement in afforestation performance can be achieved on the steppe of Central Anatolia Region holding the potential afforestation areas of Turkey, through use of forest reproductive materials from POZ and ANA stands with their higher resistance against stress, and NIK as an isolated and marginal population.

KEY WORDS: Taurus cedar, abiotic stress, peripheral population, chemical components

¹ Prof. Dr. Sezgin AYAN, Dr. Esra Nurten YER; Kastamonu University, Faculty of Forestry, Silviculture Department, Turkey.

² Dr. Nezahat TURFAN, Kastamonu University, Art & Science Faculty, Biology Department, Turkey.

³ Dr. Muhiddin ŠEHO, Bavarian Office for Forest Seeding and Planting (ASP), Teisendorf, Germany.

⁴ Assoc. Prof. Dr. Halil Barış ÖZEL, Bartın University, Faculty of Forestry, Silviculture Department, Turkey.

⁵ Dr. Fulvio DUCCI, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Forestry and Wood Research Centre (CREA FL), Arezzo, Italy. Corresponding author e.mail: sezginayan@gmail.com

INTRODUCTION

UVOD

Taurus cedar (*Cedrus libani* A. Rich.) achieves its broadest natural distribution on Taurus Mountains. The species optimum is in altitude between 800 and 2100 m. However, this altitude may descend to 530 m (in groups) and 470 m (individually) in Finike province, and reach 2400 m on Bolkar Mountains-Aydos Mountain. In addition to the main distribution of this species, isolated marginal populations are locally available near Sultandağları-Dort River, Emirdağı-Çaykışla, Tokat-Erbaa-Çatalan, Tokat-Niksar-Akıncı Village and Konya-Sağlık (Günay, 1990; Boydak, 1996).

In Central Anatolian, Taurus cedar is widely used for afforestation purposes together with Anatolian black pine (*Pinus nigra* Arnold.). This region has semi-arid climate characteristics. Fourthly percent of Turkey's lands are under this climate, and represent the main potential areas for afforestation. This increase the interest in Taurus cedar. However, differences are observed in the growth of the species depending on the local conditions (e.g. soil and climate). Additionally differences among populations were detected based on genetic parameters (Fady et al., 2008). Taurus cedar is assumed to be a frost- and drought-resistant species and is used in the afforestation practices in Turkey after some extreme events. As to last inventory data, 19 populations of cedar were selected for gene conservation and 23 populations registered as seed stands (Ayan and Yer, 2016a; Ayan et al., 2016b).

As the effects of climate change are intensely discussed, Taurus cedar is considered as a strategic, key species with its extended and variable gene pool in Turkey (approximately 500 000 ha) and its high adaptive capability. Taurus cedar is also considered as a potential species for afforestation programmes. In this regard, investigating adaptive biochemical indicators of Taurus cedar populations and detecting their genetic variation hold critical importance.

Reproductive material's quality has direct impact on the success of plantation. The most important criteria for seeds are high viability and resistance to stress in addition to physical and genetic purity (McDonald, 1999; Güney et al., 2013). Seed viability and resistance to stress are highly dependent on the seed's majority level and chemical composition in addition to genetic factors. Chemical composition of seeds basically includes carbohydrates, fats, proteins and cellulose in the membrane. Traces of compounds such as hormones, alkaloids, lectins, proteinase inhibitors, phytin and raffinose are also found in the chemical composition of seeds (Ayaz et al., 2011).

The ratio of seed chemicals in seeds also vary based on the age of the mother plant, soil characteristics, climate changes, seed harvest time, preharvest and postharvest processes and the mechanical effects arising during harvest and un-

der storage conditions (Güney et al., 2013). Insufficiencies resulting from one of the abovementioned factors effect the chemical composition of the seed, thus impairing the quality, and resulting with up to 75% reduction in the germination capability (McDonald, 2004). From this aspect, limited studies are available on seed storage chemical content of forest trees, impairment of cellular integration (lipid peroxidation- malondialdehyde (MDA)), enzymatic activities of ascorbate peroxidase (APX), guaiacol peroxidase (GuPX), catalase (CAT) and superoxide dismutase (SOD), as well as determination of α -amylase enzyme activity.

In this research; Proline, total soluble protein, MDA, Hydrogen peroxide (H_2O_2) amounts and activities of APX, GuPX, CAT, SOD and α -amylase enzymes were investigated for the seed samples of optimal and marginal Taurus cedar populations.

MATERIAL AND METHODS

MATERIJALI I METODE

Sample populations – Istraživane populacije

Seeds from five core populations and from a marginal population of Taurus cedar, used in the research, were harvested in 2016, mast seed production year. For the purpose of the research, 6 provenances selected from natural stands and representing different regions were used (Tab. 1).

Chemical Analyses – Kemijska analiza

Proline, protein, MDA and H_2O_2 in the seed samples were detected by use of the methods by Bates et al. (1973), Bradford (1976), Velikova et al. (2000). Detection of enzymatic activities in the samples was carried out by pulverization of fresh leaf sample in 0.5 g liquid nitrogen and its homogenization with 50 mM (pH 7,6) KH_2PO_4 (pH=7) 5 ml buffer solution including 0.1 mM Na-EDTA. The homogenized samples were centrifuged at +4 °C (15000 rpm) for a period of 15 minutes. Enzyme activities were analyzed in this supernatant. APX was spectrophotometrically determined using the method introduced by Nakano and Asada (1981) at 290 nm ($E=2,8$ $mM\ cm^{-1}$) by measuring the oxidation rate of the ascorbate; CAT activity was spectrophotometrically determined with the method introduced by Bergmeyer (1974); GuPX activity was detected with the modified method (Lee and Lin, 1995), and SOD enzyme activity was determined using the method applied by Cakmak et al. (2010). α -amylase activity of the seeds was calculated as the amount of starch hydrolyzed per 1 mg protein, using the method by which BSA is used as a standard (Morais and Takaki, 1998).

Statistical analyzes – Statistička analiza

The experiments were done in three replicates. Statistical analyzes of the obtained data were performed through em-

Table 1. Sample seed stand populations and their properties**Tablica 1.** Istraživane sjemenske populacije i njihove značajke

Population & Symbol / Populacija i oznaka	National Registry Number/ Nacionalni registarski broj	Latitude/ Geografska širina	Longitude/ Geografska dužina	Provenance Region Regija provenijencije	Altitude/ Nadmorska visina (m)	Exposition Ekspozicije	Age/ Dob
Antalya-Finike/Aykırıçay (FIN)	243	36° 27' 01"	30° 10' 46"	1,6	1300	N	100
Kahramanmaraş-Andırın/Elmadağı (AND)	232	37° 37' 19"	36° 28' 34"	1,1	1500	E	90
Antalya-Kaş/Karaçay (KAS)	234	36° 23' 53"	29° 26' 25"	1,6	1550	N	125
Mersin-Anamur/Abanoz (ANA)	253	36° 20' 15"	32° 56' 15"	1,4	1430	N	105
Adana-Pozantı/Pozantı (POZ)	249	37° 30' 32"	34° 57' 38"	1,2	1325	W	100
Amasya-Tokat-Niksar/Çatalan (NIK)	230	40° 47' 30"	36° 34' 40"	4,1	1100	S	100

ployment of the statistical program SPSS for Windows 20.0 Evaluation Version. Differences between control and treatment groups were analyzed through utilization of one-way ANOVA. After the variance analysis, Tukey multiple test was employed to determine differences in significance value of $P < 0,05$. In addition, the "Correlation Analysis" was performed to determine statistical relations between seed biochemical characteristics and "Spearman Correlation Coefficient" for non-normal distribution characteristics were taken into consideration.

RESULTS REZULTATI

The data related to the chemical composition and antioxidant activities of Taurus cedar seeds from different populations, have been given in table 2 and 3. According to the results of the variance analysis and multiple test results, there are significant differences between the levels of the mentioned compounds for different populations ($p < 0,05$).

Proline, total soluble protein, MDA and H₂O₂ amounts – Prolin, ukupni topivi protein, MDA i H₂O₂

Proline amounts in the sample seeds varied significantly among different populations ($p < 0,001$). The highest proline value were detected in ANA and POZ populations representing the optimum core area and the marginal-isolated NIK population from the northern distribution area. The lowest proline amount were detected in the KAS, AND and FIN population samples. As for the total soluble protein amounts, the highest values were obtained from the seed samples of FIN and KAS populations, and the lowest values were detected in ANA. High MDA concentrations were detected in NIK and AND population samples and low MDA concentrations were detected in KAS and FIN populations, two close locations. H₂O₂ concentrations in the seed samples varied between 4,68 and 11,97. The highest H₂O₂ amounts were detected in the NIK and ANA population samples (Tab. 2).

Antioxydant activities – Antioksidacijske aktivnosti

In the seed samples, APX activities varied between 0,962 Enzyme Unit (EU) and 0,248 EU. The highest APX activity was detected in POZ samples, and the lowest APX activities were detected in AND, KAS and FIN samples. The highest CAT activity was detected in POZ population (0,827 EU), whereas the lowest value was detected in FIN population (0,465 EU). ANA population exhibited the highest GuPX activity; and the seed samples of AND and KAS populations exhibited the lowest activity. The highest SOD activity was detected in the seed samples of FIN, KAS and NIK, in descending order. α -amylase activity in the seeds varied between 29,26 EU and 13,42 EU. The highest α -amylase activity was detected in the seed samples of POZ, NIK and ANA, whereas the lowest values were detected in the samples of KAS and FIN (Tab. 3).

DISCUSSION AND CONCLUSION RASPRAVA I ZAKLJUČAK

According to the literature, seed characteristics such as the seed morphology, physiology and biochemistry may vary depending on several factors such as genotype, variability of environmental factors, growth physiology of seeds, pre-harvest and postharvest processes and the interaction between these factors (McDonald, 2004). In addition to their role in the growth and development of embryo, proteins are also effective in increasing the resistance against abiotic and biotic stress factors that seedling undergoes during germination (Halliwell, 2006; Bewley et al., 2013).

In this research, the highest total soluble protein amount was detected in FIN and KAS populations, and the lowest amount was detected in ANA population (Table 2). Detection of the highest protein amount in FIN and KAS populations is attributed to low MDA concentration and high SOD activity (Foyer and Noctor, 2005; Caverzan et al., 2012). On the other hand, low protein amount in ANA samples is ascribed to high H₂O₂ concentration (Bailly, 2004; Halliwell, 2006). Also, detection of the highest proline

Table 2. Proline, total soluble protein, MDA and H₂O₂ amounts in the seed samples.Tablica 2. Prolin, ukupni topivi protein, MDA i H₂O₂ u uzorcima sjemena.

Population / <i>Populacija</i>	Proline (μmol/g)	Protein (mg/g)	MDA (μmol/g)	H ₂ O ₂ (μmol/g)
Antalya-Finike/Aykırıçay (FIN)	4,92 ± 0,04c	66,48 ± 0,18f	0,33 ± 0,001a	9,22 ± 0,28c
K.Maraş-Andırın/Elmadagı (AND)	4,37 ± 0,02b	24,77 ± 0,09c	0,41 ± 0,001c	4,68 ± 0,11a
Antalya-Kaş/Karaçay (KAS)	3,98 ± 0,02a	43,88 ± 0,24e	0,33 ± 0,001a	5,84 ± 0,09b
Mersin-Anamur/Abanoz (ANA)	7,46 ± 0,02f	11,33 ± 0,15a	0,38 ± 0,003b	11,60 ± 0,14d
Adana-Pozantı/Pozantı (POZ)	7,22 ± 0,019e	23,56 ± 0,06b	0,36 ± 0,001b	5,34 ± 0,14ab
Amasya-Tokat-Niksar/Çatalan (NIK)	5,30 ± 0,04d	30,23 ± 0,19d	0,45 ± 0,007d	11,97 ± 0,22d
<i>F Value</i>	3202,471***	13000,935***	145,517***	346,675***

Each letter shows the different homogenous groups as to multiple test results.

amount in this population is an indication of possible water deficiency stress in this region, thus increased resistance against water stress, and production of proline and glucose required for maintaining the osmotic potential (Ashraf and Foolad, 2007; Sharma et al., 2011) through protein catabolism (Tanner, 2008). This is also supported by the fact that, ANA population is in the transition zone from Mediterranean to steppe climate.

As an effective constituent in the growth and development of plants (Tanner, 2008; Verslues and Sharma, 2010) proline has an important role in the maintenance of intracellular redox balance, thus for establishment of hemostatic balance, preservation of conformational structure and form of protein, enzyme and DNA, maintenance of membrane integrity, prevention of ROS production (Ashraf and Foolad, 2007; Bhaskara et al., 2015) improvement of membrane resistance through inclusion in the secondary membrane (Verslues and Sharp, 1999; Karlsson et al., 2005), maintenance of turgor and osmotic balance (Hong et al., 2000; Gomes et al., 2010) transfer of metabolites (Kishor et al., 2005; Lehmann et al., 2010) and in physiologic processes such as germination (Hare et al., 2003). It is also important for glucose synthesis through its catabolism, and its being C, N and ATP source (Verbruggen and Hermans, 2008; Verslues and Sharma, 2010).

In the research, the highest proline amounts were detected in ANA and POZ samples. Both populations are in the transition zone from Mediterranean to steppe climate zone. The lowest proline amounts were detected in KAS and AND population samples which represent the optimal range (Table 1). The proline amount is thought to be related to and MDA content, APX, CAT, GuPX, SOD activities in AND and in MDA, GuPX, SOD KAS population. Numerous researchers suggest that proline and enzyme activities are directly effective on MDA amount (Sung, 1996; Pukacka, 1998; Sofoa et al., 2004). Detection of high proline content in POZ samples is associated with relatively high APX, CAT, GuPX and SOD activities. Detection of high proline content in POZ and ANA samples is also indicative of high wa-

ter stress (Pukacka and Ratajczak, 2005; Verslues and Sharma, 2010) or very high temperatures in these regions. Under arid conditions proline accumulates in vacuole and cytoplasm, accordingly it may have been effective in the protection of the cellular components, chemical content and water/moisture rate of the seed, as well as preservation of the morphological, physiological and biochemical structure of embryonic cells (Ueda et al., 2007; Szabados and Savoure, 2010). Moreover, higher proline and H₂O₂ amounts were associated with increased lignin synthesis in the seed testa or membrane, thus leading to an increased resistance against stress (Zhao et al., 2008; Yang et al. 2009; 2013). In the present research, detection of high proline and H₂O₂ amounts in NIK population (with an isolated and marginal distribution) and ANA population (that is in the transition zone to steppe region) is indicative of stress resistance of the seed (Table 1). In literature, proline accumulation was detected in plant tissues under stress conditions. A significant correlation between frost resistance and proline amount was also reported (Ait Barka and Audran, 1997). Proline amount significantly increased in the plants that suffer stress throughout the cold-adaptation period (Hare and Cress, 1997). Up to 3 to 6 times higher proline amounts were reported in *Citrus* sp. plants as compared to non-acclimated plants (Yelenosky, 1979).

Age of the seed, sampling time, harvest and postharvest conditions are likely to increase ROS synthesis (Bewley, 1986; Bailly, 2004; Güney et al., 2013). Oxidative stress, on the other hand, can lead to peroxidation in organelle and plasma membranes, which in turn leads to MDA accumulation (El-Maarouf-Bouteau and Bailly, 2008; Cakmak et al., 2010). In this research, the highest MDA amount was detected in NIK and AND samples, the the lowest amounts were detected in FIN and KAS samples (Table 1). High MDA content in NIK and AND seed samples was attributed to low proline amount and low activities of some of the enzymes (Bailly et al., 1996; Bhaskara et al., 2015). High H₂O₂ amount in NIK population can also be effective in increased MDA amount (Bailly, 2004; Corpas et al., 2015). Detection of the lowest MDA levels in KAS and FIN sam-

Table 3. α -amilaz, APX, CAT, GuPX and SOD activities of Taurus cedar seeds.**Tablica 3.** α -amilaza, APX, CAT, GuPX i SOD aktivnosti sjemena libanaskog cedra.

Population	APX (EU/mg Protein)	CAT (EU/mg Protein)	GuPX (EU/mg Protein)	SOD (EU/mg Protein)	α -Amilaz (EU/mg Protein)
Antalya-Finike/Aykırıçay (FIN)	0,382 \pm 0,002c	0,465 \pm 0,003a	0,357 \pm 0,001c	63,99 \pm 0,13f	13,60 \pm 0,05a
K.Maraş-Andırın/Elmadagı (AND)	0,248 \pm 0,002a	0,570 \pm 0,004b	0,232 \pm 0,004a	35,22 \pm 0,13b	15,64 \pm 0,06b
Antalya-Kaş/Karaçay (KAS)	0,358 \pm 0,002b	0,560 \pm 0,004b	0,296 \pm 0,002b	54,37 \pm 0,13e	13,42 \pm 0,04a
Mersin-Anamur/Abanoz (ANA)	0,512 \pm 0,011e	0,656 \pm 0,003c	0,473 \pm 0,003f	30,84 \pm 0,13a	16,94 \pm 0,06c
Adana-Pozantı/Pozantı (POZ)	0,962 \pm 0,005f	0,827 \pm 0,001d	0,395 \pm 0,003d	39,36 \pm 0,16c	29,26 \pm 0,06e
Amasya-Tokat-Niksar/Çatalan (NIK)	0,409 \pm 0,003d	0,480 \pm 0,006a	0,413 \pm 0,002e	43,71 \pm 0,16d	22,25 \pm 0,01d
<i>F Value</i>	2482,953***	1318,117***	1420,841***	8531,295***	9402,282***

Each letter shows the different homogenous groups as to multiple test results.

ples are indicative of very low lipid peroxidation level in these seeds (Jeng and Sung, 1994; Goel and Sheoran, 2003; Hampton et al., 2009), thus preserved membrane integrity (Demirkaya et al., 2010; Gomes et al., 2010) and protein structure (Sanders et al., 2009).

High concentrations of H₂O₂ result with oxidative stress, which may also lead to seed senescence (Vianello et al., 2007), loss in viability (Hampton et al., 2009) and morphological, biochemical degradations of seeds. H₂O₂ concentration is significantly high in ANA and NIK samples of Taurus cedar. The lowest H₂O₂ levels, on the other hand, were detected in AND and KAS seed samples (Table 1). The varying H₂O₂ content is primarily ascribed to the differences in the growth environments (Jeng and Sung, 1994; Güney et al., 2013). Also the detection of low H₂O₂ content in the locations with high enzymatic activities is associated with the function of CAT, GuPX and APX activities in inhibition of ROS synthesis (Lehner et al., 2008).

Seeds contain enzymes in addition to carbohydrates, fats, hormones, minerals, proteins and aminoacids (Hare et al., 2003; Bewley et al., 2013). These compounds protect cells, tissues and organs through preventing the damages induced by ROS and lipidperoxidation, or repairing damaged tissues, or suppressing ROS synthesis (Bailly et al., 2000).

According to the enzymatic activity values obtained from Taurus cedar population samples, the highest APX and CAT enzyme activities were detected in POZ, the highest GuPX activity was detected in ANA and NIK samples, and the highest SOD enzyme activity was detected in FIN, KAS and NIK samples in descending order (Table 3). Various researchers reported that, APX and CAT enzymes inhibit H₂O₂ and ROS synthesis, thus reducing the possible damages of oxidative stress (Bailly, 2004). However, low MDA and high proline and protein contents were detected in these seeds. This finding is indicative of the completion of tissue differentiation and the necessity of H₂O₂ inhibition for testa or other tissues' development (Corpas et al., 2015). It was verified in previous researches that hydrolytic enzymes released during seed development lead to peroxida-

tion in cell and organelle membranes, thus resulting with increased MDA accumulation (McDonald, 2004). Low APX and GuPX activities in KAS samples led to reduced levels of H₂O₂ and MDA (Jeng and Sung, 1994; Caverzan et al., 2012). Protein amount was also affected by these enzymatic activities (Palma et al., 2002). The decrease in the protein content may also have stemmed from the increased proline amount. It was detected in previous researches that, proline amount triggered protein catabolism under arid and low temperature conditions (Hare et al., 2003; Sanders et al., 2009).

Varying enzyme activities in the seed samples are primarily ascribed to varying locations, thus climate and soil characteristics (Ertekin et al., 2015), seed storage conditions (Pukacka and Ratajczak, 2005; Pekşen and Palabıyık, 2013), and harvesting method. As indicated by the results of literature studies and evaluation of the obtained data, activities of the enzymes such as SOD, CAT; APX and GuPX can be used as indicators in the determination of seed quality (McDonald, 1998; Corbıneau, 2012).

In addition to antioxidant enzymes, seeds also contain enzymes that control germination (Schmidt et al., 2007). It was reported in literature studies on seed germination that, seeds contain amylase (α and β) enzymes (Black et al., 1996) that breakdown starch into glucose, fructose and sucrose (Cochrane et al., 2000; Palma et al., 2002). The lowest α -amylase activities were detected in KAS and FIN samples, and the highest activities were detected in POZ and NIK samples of Taurus cedar populations (Table 2). According to numerous researchers, seed storage attributes are dependent on climate changes, soil characteristics, age and size of tissues and organs, and competitiveness (Price et al., 2003; Lehner et al., 2008).

The signal transduction and responses against stress conditions vary in roots and leaves of plants. H₂O₂ is effective in signal transduction and antioxidant enzyme activities. Plants are equipped with antioxidant defense system that control ROS levels; and this system comprise of defending elements such as SOD, CAT, GuPX, APX and The plants

have an antioxidant defense system that will control the levels of ROS and this system; SOD, CAT, GuPX, APX, glutathione reductase (GR).

From the practical point of view, improvement in afforestation performance and possibly adaptation can be achieved in the steppe of Central Anatolia Region holding the potential afforestation areas of Turkey, through the use of reproductive material from POZ and ANA populations, that represent the main distribution of Taurus cedar in the transition zone from Mediterranean to steppe climate, with higher resistance against water deficiency stress, and NIK as an isolated and marginal population.

ACKNOWLEDGEMENT

ZAHVALA

As a non-project study, this research was carried out using the facilities of projects no KÜBAP-01/2013-17, 01/2013-59, and 01/2014-21. The seed samples were supplied from General Directorate of Forestry (GDF). We acknowledge the support and contributions of Kastamonu University and GDF. We are also thankful for the supports of my M.Sc. students Asuman TAN and Halit ÇELİKBAŞ in laboratory studies.

REFERENCES

LITERATURA

- Ait Barka, E., J. C. Audran, 1997: Response of champenoise grapevine to low temperatures: Changes of shoot and bud proline concentrations in response to low temperatures and correlations with freezing tolerance. *J. Hortic. Sci. Biotechnol.* 72:577–582.
- Ashraf, M., M.R. Foolad, 2007: Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Exp. Bot.* 59 (2): 206–216.
- Ayan, S., E.N. Yer, 2016a: Assessment of Taurus cedar (*Cedrus libani* A. Rich) plantations out of their natural distribution areas in Turkey with regards to ecological factors. Abstract Book, p. 10, Résumés du colloque international Sous le thème: «Les espaces forestiers péri-forestiers (EFPF): dynamique et défis». Les 3-5 novembre 2016 Campus Universitaire Ait Melloul-Université IBN Zohr-Agadir, Morocco.
- Ayan, S., Erkan B.S., Yer, E.N., E. Buğday, 2016b: Populations Characteristics of *Cedrus libani* A. Rich. natural forests in Turkey. Abstract Book p. 50, Résumés du colloque international Sous le thème: «Les espaces forestiers péri-forestiers (EFPF): Dynamique et défis». Les 3-5 novembre 2016 Campus Universitaire Ait Melloul-Université IBN Zohr-Agadir, Morocco.
- Ayaz, F.A., Glew, R.H., Turna, I., Güneş, D., Chuang, L.T., Chang, Y.C., Andrews, R., Power, L., Presley, J., Torun, H., N. Sahin, 2011: *Fagus orientalis* (Oriental beech) seeds are a good source of essential fatty acids, amino acids and minerals. *Food* 5: 48–51.
- Bailly, C., 2004: Active oxygen species and antioxidants in seed biology. *Seed Science Research* 14: 93–107.
- Bailly, C., Benamar, A., Corbineau, F., D. Come, 2000: Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Sci. Res.* 10: 35–42.
- Bailly, C., Benamar, A., Corbineau, F., D. Come, 1996: Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiologia Plantarum* 97: 104–110.
- Bates, L., Waldern, R.P., I.D. Teare, 1973: Rapid determination of free proline for water-stress studies. *Plant and Soil* 39: 205–207.
- Bergmeyer, H.U., 1974: Principles of enzymatic analysis. In *Methods of enzymatic analysis*, ed. Bergmeyer H.U. Academic Press, New York, p. 94.
- Bewley, J.D., 1986: Membrane changes in seeds as related to germination and perturbations resulting from deterioration in storage. In: M. B. Jr. McDonald, and C. J. Nelson (Eds.) *Physiology of Seed Deterioration* Madison, Crop Science Society of America. Inc. P: 27–45.
- Bewley, J.D., Bradford, K., Hillhorst, H.W.M., H. Nonogaki, 2013: *Seeds Physiology of Development, Germination and Dormancy*. Springer: New York.
- Bhaskara, G.B., Yang, T.H., P.E. Verslues, 2015: Dynamic proline metabolism: importance and regulation in water limited environments. *Frontiers in Plant Science* 6 - doi: 10.3389/fpls.2015.00484.
- Black, M.C., Ferrell, J., Horning, R.C., L.K. Martin, 1996: DNA strand breakage in freshwater mussels (*Anodonta grandis*) exposed to lead in the laboratory and field. *Environmental Toxicology and Chemistry*. 15 (5) 802–808.
- Boydak, M., 1996: Ecology and Silviculture of Taurus cedar (*Cedrus libani* A. Rich.) and Preservation of Natural Forests. Ministry of Forestry Publication No: 012, 78p. Ankara.
- Bradford, M.M., 1976: A rapid sensitive method for the quantitation of micro program quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254.
- Cakmak, T., Atici, O., S. Sunar, 2010: Natural aging related biochemical changes in alfalfa (*Medicago sativa* L.) seeds stored for 42 years. *Int Res J Plant Sci.* 1:1–6.
- Caverzan, A., Passaia, G., Rosa, S.B., Ribeiro, C.W., Lazzarotto, F., M. Margis-Pinheiro, 2012: Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genet. Mol. Biol.* 35: 1011–1019.
- Cochrane, M.P., Paterson, L., E. Gould, 2000: Changes in chlamydomonas cell walls and in the peroxidase enzymes of the crease region during grain development in barley. *J. Exp. Bot.* 51: 507–520.
- Corbineau, F., 2012: Markers of seed quality: from present to future. *Seed Science Research* 22:61–68.
- Corpas, F.J., Gupta, D.K., J.M. Palma, 2015: Production sites of reactive oxygen species (ROS) in organelles from plant cells. In *Reactive Oxygen Species and Oxidative Damage in Plants under Stress*, pp.1–22. Springer International Publishing.
- Demirkaya, M., Dietz, K.J., H.O. Sivritepe, 2010: Changes in antioxidant enzymes during ageing of onion seeds. *Notulae Bot. Horti Agrobotanici Cluj-Napoca* 38:49–52.
- El-Maarouf-Bouteau, H., C. Bailly, 2008: Oxidative signalling in seed germination and dormancy. *Plant Signal Behav.* 3:175–182.
- Ertekin, M., Kırdar, E., S. Ayan, 2015: Effects of tree ages, exposures and elevations on some seed characteristics of Oriental beech (*Fagus orientalis* Lipsky.). *SEEFOR - South-east European Forestry* 6 (1): 15–23.
- Fady, B., Lefèvre, F., Vendramin, G.G., Ambert, A., Règnier, C., M. Bariteau, 2008: Genetic consequences of past climate and human impact on eastern Mediterranean *Cedrus libani* forests. Im-

- plications for their conservation. *Conservation Genetics* 9: 85-95.
- Foyer, C.H., G. Noctor, 2005: Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell* 17:1866-1875.
 - Goel, A., I.S. Sheoran, 2003: Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. *Biol Plant*. 46:429-434.
 - Gomes, F.P., Oliva, M.A., Mielke, M.S., Almeida, A.A.F., L.A. Aquino, 2010: Osmotic adjustment, proline accumulation and cell membrane stability in leaves of *Coccosnucifera* submitted to drought stress. *Scientia Horticulturae* 126 (3): 379-384.
 - Günay, T. 1990: Afyon - Emirdağ Yukarı Çaykışla Vadisi'nde Stepe Geçiş Kuşağında Yeni Tespit Edilen Bir Sedir (*Cedrus libani* A. Rich) Kalıntı Mesçeresi ve Ekolojik Özellikleri. *Uluslararası Sedir Sempozyumu* p. 53-63. Antalya.
 - Güney, D., Bak, Z.D., Aydınoğlu, F., Turna, I., F.A. Ayaz, 2013: Effect of geographical variation on the sugar composition of the oriental beech (*Fagus orientalis* Lipsky). *Turk J Agric For*: 7: 221-230.
 - Halliwell, B., 2006: Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol*. 141: 312-322.
 - Hampton, J.G., Leeks, C.R.F., B.A. McKenzie, 2009: Conductivity as a vigour test for *Brassica* species. *Seed Science and Technology*, 37: 214-221.
 - Hare P.D., W.A. Cress, 1997: Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regulation* 21, 79-102.
 - Hare, P.D., Cress, W.A., J. Van Staden, 2003: A regulatory role for proline metabolism in stimulating *Arabidopsis thaliana* seed germination. *Plant Growth Regul* 39:41-50.
 - Hong, Z., Lakkineni, K., Zhang, Z., D.P.S. Verma, 2000: Removal of feedback inhibition of $\Delta 1$ -pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiology* 122 (4):1129-1136.
 - Jeng, T.J., J.M. Sung, 1994: Hydration effect on lipid peroxidation and peroxide scavenging enzymes activity of artificially age peanut seed. *Seed Sci Tech*. 22:531-539.
 - Karlsson, M., Melzer, M., Prokhorenko, I., Johansson, T., G. Wingsle, 2005: Hydrogen peroxide and expression of hipl-superoxide dismutase are associated with the development of secondary cell walls in *Zinnia elegans*. *J. Exp. Bot*. 56: 2085-2093.
 - Kishor, K.P.B., Sangam, S., Amrutha, R.N., Laximi P.S., Naidu, K.R., Rao, K.R.S., Rao, S., Reddy, K.J., Theriappan, P., N. Sreenivasulun, 2005: Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Current Science* 88 (3): 424-438.
 - Lee, T.M., Y.H. Lin, 1995: Changes in soluble and cell wallbound peroxidase activities with growth in anoxia-treated rice (*Oryza sativa* L.) coleoptiles and roots. *Plant Sci*. 106:1-7.
 - Lehmann, S., Funck, D., Szabados, L., D. Rentsch, 2010: Proline metabolism and transport in plant development. *Amino Acid* 39 (4):949-962.
 - Lehner, A., Mamadou, N., Poels, P., Come, D., Bailly, C., F. Corbineau, 2008: Change in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. *J. Cereal Sci*. 47: 555-565.
 - McDonald MB. 2004: Orthodox seed deterioration and its repair, pp. 273-304. In: *Handbook of Seed Physiology: Applications to Agriculture*, Benech-Arnold, R. L. and R.A. Sanchez (Eds.). Food Products Press, New York.
 - McDonald, M.B., 1998: Seed quality assessment. *Seed Science Research*. 8:265-275.
 - McDonald, M.M., 1999: Seed deterioration: physiology repair and assessment. *Seed Sci Tech*. 27:177-237.
 - Morais, G., M. Takaki, 1998: Determination of amylase activity in cotyledons of *Phaseolus vulgaris* L. cv. *carrioca*. *Brazilian Archives of Biology and Technology* 41: 17-25.
 - Nakano, Y., K. Asada, 1981: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*. 22 (5): 867-880.
 - Palma, J.M., Sandalino, L.M., Corpas, F.J., Romero-Puertas, M.C., McCharty, I., L.A. Del Rio, 2002: Plant Proteases, Protein Degradation, and Oxidative Stress: Role of Peroxisomes. *Plant. Physiol. Biochem*. 40: 521-530.
 - Pekşen, E., B. Palabıyık, 2013: Effect of Seed Storage on Seed Yield and Yield Related Characteristics of Common Bean Grown in Different Environments, Iğdır University, *Journal of Institute of Science and Technology*. 3 (2): 93-102.
 - Price, J., Li, T.C., Kang, S.G., Na, J.K., J.C. Jang, 2003: Mechanisms of glucose signaling during germination of *Arabidopsis*. *Plant Physiol*. 132: 1424-1438.
 - Pukacka, S., E. Ratajczak, 2005: Production and scavenging of reactive oxygen species in *Fagus sylvatica* seeds during storage at varied temperature and humidity. *J. Plant Physiol*. 162: 873-885.
 - Pukacka, S., 1998: Changes in membrane fatty acid composition during desiccation of seeds of silver maple. *Seed Sci. and Technol*. 26: 535-540.
 - Sanders, A., Collier, R., Trethewy, A., Gould, G., Sieker, R., M. Tegeder, 2009: AAP1 regulates import of amino acids into developing *Arabidopsis* embryos. *Plant J* 59:540-552.
 - Schmidt, R., Stransky, H., W. Koch, 2007: The amino acid permease AAP8 is important for early seed development in *Arabidopsis thaliana*. *Planta* 226:805-813.
 - Sharma, S., Villamor, J.G., P.E. Versules, 2011: Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. *Plant Physiology* 157(1):292-304.
 - Sofoa, A., Dichioa, B., Xiloyannisa, C., A. Masiab, 2004: Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. *Physiologia Plantarum* 121:58-65.
 - Sung, J.M. 1996: Lipid peroxidation and peroxide scavenging in soybeans seeds during aging. *Physiol Plant* 97:85-89.
 - Szabados, L., A. Savoure, 2010: Proline: a multifunctional amino acid. *Trends Plant Sci* 15:89-97.
 - Tanner, J., 2008: Structural biology of proline catabolism. *Amino Acids* 35:719-730.
 - Ueda, A., Yamamoto-Yamane, Y., T. Takabe, 2007: Salt stress enhances proline utilization in the apical region of barley roots. *Biochem. Biophys. Res. Commun* 355:61-66.
 - Velikova, V., Yordanov, I., A. Edrava, 2000: Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci*. 151: 59-66.

- Verbruggen, N., C. Hermans, 2008: Proline accumulation in plants: a review. *Amino Acids* 35:753-759.
- Verslues, P.E., R.E. Sharp, 1999: Proline accumulation in maize (*Zea mays* L.) primary roots at low potentials. Metabolic source of increased proline deposition in the elongation zone. *Plant Physiology* 119 (4):1349-1360.
- Verslues, P.E., S. Sharma, 2010: Proline metabolism and its implications for plant-environment interaction. *Arabidopsis Book* 8 (3) - doi: 10.1199/tab.0140.
- Vianello, A., Zancani, M., Peresson, C., Petrusa, E., Casolo, V., Krajňáková, J., F. Macrì, 2007: Plant mitochondrial pathway leading to programmed cell death. *Physiol. Plant.* 129: 242-252.
- Yang, F., Mitra, P., Zhang, L., Prak, L., Verhertbruggen, Y., Kim, J.S., D. Loqué, 2013: Engineering secondary cell wall deposition in plants. *Plant Biotechnol J.*, 11: 325-335.
- Yang, S.L., Lan, S.S., M. Gong, 2009: Hydrogen peroxide-induced proline and metabolic pathway of its accumulation in maize seedlings. *J Plant Physiol.* 166:1694-1699.
- Yelenosky, G., 1979: Accumulation of Free Proline in Citrus Leaves during Cold Hardening of Young Trees in Controlled Temperature Regimes. *Plant Physiology.* 64 (3) 425-427.
- Zhao, C., Avci, U., Grant, E.H., Haigler, C.H., E.P. Beers, 2008: XND1, a member of the NAC domain family in *Arabidopsis thaliana*, negatively regulates lignocellulose synthesis and programmed cell death in xylem. *Plant J* 53, 425-436.

SAŽETAK

Genetska raznolikost je osnova za prilagodbu i opstanak vrsta drveća u promjenjivim uvjetima okoline te predstavlja ključni uvjet stabilnosti i produktivnosti šumskih ekosustava. Predmet ovog istraživanja su glavne i marginalne populacije libanonskog cedra (*Cedrus libani* A. Rich.) zbog njihove važnosti u očuvanju gena. Procjena genetske varijabilnosti u izoliranim populacijama od velike je važnosti za programe očuvanja i poboljšanja. U uvjetima djelovanja globalnih klimatskih promjena populacije mogu posjedovati genotipove budućeg prilagodljivog potencijala. Cilj ove studije je utvrditi: (a) količinu prolina, za bolje razumijevanje stresa uzrokovanog nedostatkom vode u populaciji, (b) ukupnih topljivih proteina, MDA, H2O2, α -amilaze te (c) varijabilnosti antioksidansa kao CAT, SOD, APX i GuPX kod sjemena pet glavnih populacija (AND-Kahramanmaraş-Andırın/Elmadağ, POZ-Adana-Pozanti/Pozanti, ANA-Mersin-Anamur/Abanoz, FIN-Antalya-Finike/Aykırıçay and KAS-Antalya-Kaş/Karaçay) i jedne marginalne populacije Amasya-Tokat-Niksar/Çatalan (NIK) u Turskoj. Prema dobivenim rezultatima, značajna razlika je otkrivena među populacijama. Značajno veće količine prolina otkrivene su za populaciju ANA (7,46 $\mu\text{mol/g}$) i POZ (7,22 $\mu\text{mol/g}$), dok su najniže količine prolina otkrivene u populaciji KAS (3,98 $\mu\text{mol/g}$) koje predstavljaju optimalnu distribuciju libanonskog cedra. Ovaj rezultat pokazuje da su populacije POZ i ANA, u prijelaznoj zoni iz mediteranske regije do područja stepa, otpornije na mraz nego druge populacije. Najveća količina enzima α -amilaze detektirana je u POZ populaciji koja raste u optimalnom rasprostriranju libanonskog cedra. Značajno više razine H₂O₂ detektirane su u populacijama NIK (11,97 $\mu\text{mol/g}$) i ANA (11,60 $\mu\text{mol/g}$), što ukazuje na veće razine oksidacijskog stresa u uzorcima sjemena iz tih populacija. Ovim istraživanjem potvrđeno je da su enzimi poput SOD, CAT, GuPX i APX koji kontroliraju razine reaktivnih vrsta kisika (ROS) u biljnim stanicama, elementi antioksidacijskog obrambenog sustava koji djeluju kao zaštitni mehanizmi biljaka protiv stresnih stanja. S praktičnog gledišta, poboljšanje pošumljavanja može se postići na stepi Srednje Anadoljske regije koja posjeduje značajane površine za pošumljavanje u Turskoj, primjenom šumskog reprodukcijskog materijala iz populacija POZ i ANA koje pokazuju veću otpornosti na stres, kao i populacije NIK koja je izolirana i marginalna.

KLJUČNE RIJEČI: libanonski cedar, abiotički stres, rubna populacija, kemijski sastav