Relationship between Seed Germination and Catalase Enzyme Activity of *Abies* taxa from Turkey

***Ayşe AK[1], Ersin YÜCEL[1], Sezgin AYAN[2]**
[1]Anadolu University, Science Faculty, Biology Department Eskişehir / TURKEY
[2]Kastamonu University, Forest Faculty, Silviculture Department Kastamonu / TURKEY
*Corresponding Author: a_ak@hotmail.com

Abstract

Reactive oxygen species (ROS) are involved in various aspects of seed physiology. Their generation, which occurs during seed desiccation, germination and ageing, may lead to oxidative stress and cellular damage, resulting in seed deterioration. However, cells are endowed with detoxifying enzymes and antioxidant compounds that scavenge ROS and participate in seed survival. The detoxifying mechanisms play a key role in completion of seed germination and seed storability. The enzyme catalase has been employed for determining viability of seeds. The plan of the present work was to explore the relationship between catalase activity and germination of four *Abies* taxa. For this purpose, seeds which belong to four *Abies* taxa (*Abies nordmanniana* subsp. *equi-trojani*, *A. cilicica*, *A. nordmanniana* ssp. *bornmuelleriana*, *A. nordmanniana* ssp. *nordmanniana*) collected from different provenances in 2010 and 2011 harvesting years were used. Germination percentages and catalase enzyme activities of different *Abies* taxa were measured. Our results demonstrate that *Abies* seeds had different germination percentage and catalase enzyme activity. *Abies nordmanniana* subsp. *equi-trojani* seeds which are collected from Edremit-Gürgendag have higher germination percentage and catalase activity ($P<0.05$). Other populations of *Abies* did not differ from each other. In a conclusion, high activity of catalase enzyme refers to viability and quality of seed in *Abies* taxa.

Keywords: *Abies* taxa, Germination, Catalase enzyme activity

Introduction

Abies is the second largest genus of family Pinaceae after *Pinus*, and includes 51 species (Liu, 1971). *Abies* species occur in the highlands of Asia, Europe, North and Middle America, and North Africa (Yang et al., 2008). *Abies* species are economically valuable because of many features like timber, paper pulp, oils and resins. In addition, some species are used as ornamentals (Anşin and Özkan, 1997; Esteban et al., 2010). There are six native *Abies* taxa growing in pure and mixed stands in Turkey. These are *Abies cilicica* subs. *isaurica*, *Abies cilicica* subsp. *cilicica*, *Abies nordmanniana* subsp. *bornmüelleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies nordmanniana* subsp. *nordmanniana* and *Abies x olcayana*. Four of these taxa, *Abies cilicica* subs. *isaurica*, *Abies nordmanniana* subsp. *bornmuelleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies x olcayana* are endemic and considered as low risk (LR) species according to IUCN criteria (Ekim et al., 2000). Firs are generally considered a group of species exhibiting great variability in morphological, anatomical and biochemical traits (Scaltsoyiannes et al., 1999). Fir seeds are usually low quality because of dead seed or abiotic and biotic factors such as insect, outbreaks, climatic conditions, storm and windfalls (Kolotelo, 1998). Seed is the first starting point of plantation. Therefore it is important to select and use high quality seed with good physiological, biochemical and phyto-pathological properties for forestry activities (Milosevic et al., 2010). Knowledge of seed is required for the success of plantations (Khurana and Singh, 2001). Seed viability is measured to maintain the germination efficiency of stored seeds and has economical implications (Demirkaya et al., 2010). Genetic and environmental factors influence seed growth and development. When environmental factors exceed tolerance of a plant, reactive oxygen species (ROS) are generated in the cell (Cai et al., 2011; Chau et al., 2011; Oprica, 2008). ROS affect various aspects of seed physiology. Antioxidants which act as ROS scavenger in seed biology play a very important role in the growth processes during seed development. Some protective mechanisms involving ROS scavenging enzymes, such as catalase (CAT), have been evaluated within the mechanism of seed (Ishibashi et al., 2008). Studies of relationship between antioxidative enzymes and germination of coniferous trees are rare.

The aim of present study is to investigate the relationship between germination and catalase activity of *Abies* taxa form Turkey.

Material and Method

Seeds of four *Abies* taxa were collected from different provenances of Turkey. All data which include code and locations of population and harvest year were presented in Table 1.

Each germination experiment comprised experimental series of 3 x 25 seeds. In the climate chamber, filter-papers were used and a constant temperature (23±ºC) was maintained throughout the experiment. Germination tests were performed with the three replicates in a Petri dish (9 cm diameter lined with two discs of filter paper) under dark conditions.

Table 1. Studied seeds of *Abies* population

Population code	Taxon	Population	Harvest year
Caucasian Fir (CF1)	*Abies nordmanniana* subsp. *nordmanniana* (Steven) Spach	Koyulhisar-Sisorta	2010
Caucasian Fir (CF2)	*Abies nordmanniana* subsp. *nordmanniana* (Steven) Spach	Şavşat-Yayla	2011
Kazdağı Fir (KF1)	*Abies nordmanniana* subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen.	Çan-Çan	2010
Kazdağı Fir (KF2)	*Abies nordmanniana* subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen.	Edremit- Gürgendağ	2010
Toros Fir (TF)	*Abies cilicica* (Ant. et Kotschy) Carr.	Pozantı- Ulukışla	2010
Bornmüllerian Fir (BF1)	*Abies nordmanniana* subsp. *bornmülleriana* Mattf.	Akyazı-Dokurcun	2011
Bornmüllerian Fir (BF2)	*Abies nordmanniana* subsp. *bornmülleriana* Mattf.	Bolu-Kökez	2011
Bornmüllerian Fir (BF3)	*Abies nordmanniana* subsp. *bornmülleriana* Mattf.	Bursa-Uludağ MP	2011

For enzyme extraction, 0.3 gr embriyos were homogenized in extraction buffer 50 mM sodium phosphate buffer (pH 7.6) containing 10 mM EDTA and 10% (w/v) PVPP. Homogenates were centrifuged at 12,000 g for 15 minutes. Catalase activity (unit.mg^{-1}) was spectrophotometrically determined. The decomposition of H_2O_2 was monitored by the decrease in absorbance at 240 nm in a reaction mixture that contained 50 mM potassium phosphate buffer (pH 7.0), the sample and 10 mM H_2O_2. The assay was performed at 25 °C in a 3 ml cuvette. The protein concentration was measured according to Bradford (1976) using bovine serum albumin as the standard.

Data were subjected to analysis of variance (ANOVA) and with significant differences between means identified by Duncan's multiple range ($P<0.05$). To compare seed germination or catalase activity for two years (2010 and 2011), paired data were analyzed using Student's t test.

Results

As a result of analysis of variance on germination percentage, differences were observed between populations ($P<0.05$). The highest germination percentage with 78% was determined from seeds of population KF2 which were collected from Edremit-Gürgendağ (Table 2).

Table 2. Duncan test results on percentage of germination in populations

Population	N	Germination proportion (%)		
BF1	3	39		
BF2	3	41		
BF3	3	44	44	
TF	3	45	45	
CF2	3	46	46	
CF1	3	50	50	
KF1	3		55	
KF2	3			78
Sig.		0.081	0.062	1.000

Germinations of *Abies* taxa were different. This difference was highly significant. The highest germination percentage was observed in seeds of *A. nordmanniana* subsp. *equi-trojani* (KF) which originated from Çan-Çan and Edremit-Gürgendağ in the 2010 seed harvest year ($P<0.05$) (Table 3).

Table 3. Duncan test results on percentage of germination in *Abies* taxa

Taxa	N	Germination proportion (%)	
BF	9	41	
TF	3	45	
CF	6	48	
KF	6		66
Sig.		0.282	1.000

Seeds of *Abies* populations from different region of Turkey were collected in 2010 and 2011. The germination percentage of seeds

collected in 2010 was higher than of seeds collected in 2011 (Table 4).

Table 4. Germination proportions of seeds collected in 2010 and 2011

Germination proportion (%)		N	Mean	Std. Deviation	Std. Error Mean
Harvest year	2010	12	57.25	14.09142	4.06784
	2011	12	42.91	5.79119	1.67177

CAT activity (unit.mg^{-1}) was significantly different between populations. Two homogeneous groups were statistically different (*P<0.05*). KF2 had more activity of CAT when compared to other populations (Table 5).

Table 5. CAT activity in *Abies* populations

Population	N	CAT activity	
BF3	3	181.00	
BF2	3	225.06	
CF2	3	256.72	
BF1	3	257.40	
CF1	3	258.39	
KF1	3	273.13	
TF	3	286.41	
KF2	3		437.93
Sig.		0.053	1.000

When catalase activities of *Abies* taxa were observed, two homogeneous groups were statistically different (*P<0.05*). *A. nordmanniana* subsp. *equi-trojani* (KF) and *Abies cilicica* (Ant. et Kotschy) Carr. (TF) had similar CAT activity (Table 6).

Table 6. CAT activity of *Abies* taxa

Taxa	N	CAT activity	
BF	9	221.15	
CF	6	257.56	
TF	3	286.41	286.41
KF	6		355.53
Sig.		0,171	0,129

As shown in Table 7, CAT activity of seeds collected in 2010 was higher than of seeds collected in 2011.

Table 7. CAT activity of seed collected in 2010 and 2011

CAT activity	Year	N	Mean	Std. Deviation	Std. Error Mean
Harvest year	2010	12	313.969	78.59216	22.68760
	2011	12	230.049	70.40547	20.32431

Discussion and Conclusion

Germination is a very complex process, and is affected by many factors (Shafii and Price, 2001). Minimum accumulation of ROS and enhanced activity of enzymes affect seed quality thus germination potential (Rao et al., 2006). In addition to optimal environmental conditions, seed must be of quality for the beginning of germination (McDonald, 1998). One of the factors that determine seed quality is the presence of catalase enzyme (Bailly et al., 2001). Antioxidant enzyme catalase which allows the tolerance of plant under stress conditions is also claimed to be effective in the physiology of germination (Scandalios, 1993; Reuzeau and Cavalie 1995; Bailly, 2004; Kibinza et al., 2011). CAT activity in seeds and seedlings is also involved in preservation of viability during storage and necessary for seed germination and early seedling growth (Bernal-Lugo et al., 2000; Milosevic et al., 2010). Catalase activity in seeds may serve as a parameter that indicates the germination capacity (Prodanovic et al., 2007). There are several studies about changes of enzyme activity that is related to germination, development and tolerance of plant (Bailly et al., 2001; Cai et al., 2011). It was shown that germination is closely related with the catalase activity (Jihong and Qing, 2009; Demirkaya et al., 2010).

In conclusion, different percentage of germination and catalase enzyme activity were obtained from seeds of *Abies nordmanniana* subsp. *equi-trojani* collected from Edremit-Gürgendağ in 2010, when compared to other *Abies* populations. Although these differences may be explained by having different genetic material, germination and catalase activity of *Abies* subsp. *equi-trojani* population from Çan-Çan was lower than that collected from Edremit-Gürgendağ. Collected from different locations and harvest year or different seed storage conditions may also cause losses of seed viability. Regardless of the outcome, the viability and quality of seed are linked to the activity of catalase enzyme. We conclude that seeds of *Abies nordmanniana* subsp. *equi-trojani* have higher viability because of having higher catalase activity. Measurement of catalase activity may be a parameter to determine seed viability and germination.

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