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Research Article

CHANGES IN PROTEIN AND SUGAR CONTENTS DURING THE GERMINATION PERIOD OF TURKISH RED PINE (*Pinus brutia* Ten.)

Fatih Tonguç[1,*,a], Muhammet Tonguç[2,b], Sercan Önder[2,c]

[1]Applied Sciences University of Isparta, Faculty of Forestry, Dept. of Forest Engineering, Isparta, Turkey
[2]Applied Sciences University of Isparta, Faculty of Agriculture, Dept. of Agricultural biotechnology, Isparta, Turkey

ORCID ID[a]: 0000-0002-0820-4820 ORCID ID[b]: 0000-0003-1292-2910
ORCID ID[c]: 0000-0002-8065-288X

[*]Corresponding Author: fatihtonguc@isparta.edu.tr

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Abstract

Pinus brutia Ten., also known as Turkish red pine, belongs to Pineceae family and is an important forest species for afforestation operations and covers approximately 25% of forests in Turkey. It is naturally distributed in Eastern Mediterranean countries, and it forms natural stands in Mediterranean, Aegean, Black Sea and Marmara regions in Turkey. High quality seeds are very important for growth and seedling establishment for plant species. One of the measurements of the seed quality is chemical composition of the seeds. In the present study, Turkish red pine (Pinus brutia Ten.) seeds were germinated for 25 days. Changes were investigated for protein and sugar contents during the germination and early seedling growth. Significant differences were observed between different growth periods for soluble protein and sugars contents.

Keywords: Chemical composition; germination; Pinus brutia; seed.

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Arastirma Makalesi

KIZILÇAMDA (*Pinus brutia* Ten.) ÇİMLENME DÖNEMİ BOYUNCA PROTEİN VE ŞEKER İÇERİĞİNDEKİ DEĞİŞİMLER

Özet

Türk kızılçamı olarak da bilinen Pinus brutia Ten., Pineceae familyasına aittir ve ağaçlandırma operasyonları için önemli bir orman ağacıdır ve Türkiye'deki ormanların yaklaşık % 25'ini kapsamaktadır. Doğal olarak Doğu Akdeniz ülkelerine dağılmıştır ve Türkiye'nin Akdeniz, Ege, Karadeniz ve Marmara bölgelerinde doğal yayılış gösterir. Bitki türlerinde yüksek kaliteli tohumlar tohum çimlenmesi ve fide gelişmi için çok önemlidir. Tohum kalitesini etkileyen kıstaslarından birisi de tohumların ihtiva ettiği kimyasal maddelerin içeriğidir. Mevcut çalışmada kızılçam (Pinus brutia Ten.) tohumları 25 gün boyunca çimlendirilmiştir. Çimlenme ve erken fide gelişimi boyunca protein ve şeker içeriklerindeki değişimler incelenmiştir. Farklı çimlenme ve gelişim dönemleri boyunca protein ve şeker içeriklerinde önemli farklılıklar olduğu çalışma sonucu bulunmuştur.

Anahtar Kelimeler: Kimyasal içerik, çimlenme, *Pinus brutia*, tohum.

1. INTRODUCTION

Pinus brutia Ten., also known as Turkish red pine, belongs to Pineceae family and is an important forest species for afforestation operations and covers approximately 25% of forests in Turkey. It is naturally distributed in Eastern Mediterranean countries, and it forms natural stands in Mediterranean, Aegean, Black Sea and Marmara regions in Turkey (Boydak et al., 2006; OGM, 2015).

Pollination and fertilization are important biological activities in plant lives as they are necessary steps to produce seeds. Plant seeds accumulate different metabolites such as; storage proteins, lipids and carbohydrates, necessary for seed germination. Stored reserves are used as sources of energy and primary metabolites during the germination and early seedling growth until seedlings establish themselves to begin photosynthesis to produce their own sugars (Bewley and Black, 1994). Red pine seeds reach anatomical maturation two years after the pollination and fertilization (Eler and Şenergin, 1990). Seed dispersal begins at the third year after the pollination and fertilization and continues throughout the year (Boydak et al., 2006). Little is known about the chemical composition of the Pinus species, its metabolism during the germination and its influence on seedling growth. Pine species contain 22-68% lipid in their seeds and the red pine seeds oil content is 23% (Wolff and Bayard, 1995; Bağcı and Karaağaçlı, 2004). The starch and protein contents of red pine seeds have not been investigated, P. halepensis seeds contain 31% protein and 14% carbohydrates (Nergiz and Dönmez, 2004).

Red pine seeds could germinate ranging from 5-25 °C, but the optimum temperature for germination is between 15-20 °C (Scordilis and Thanos, 1995). Different seed germination studies with red pine have been reported for seed production (Ürgenç et al., 1989), dormancy and dormancy breakdown treatments (Thanos and Skordilis, 1987; Skordilis and Thanos, 1995; Boydak et al., 2006), germination performance of seeds from different populations (Boydak et al., 2003; Tilki and Dirik, 2007; Yılmaz et al., 2013), germination and seedling characteristics of different populations (Işık, 1986).

Physiological changes during the germination and early seedling growth has been investigated for many crop species such as; sunflower (Erbaş et al., 2016), mung bean, pea and lentil (El-Adawy et al., 2003), oat (Leonova et al., 2010). There is a growing interest to investigate germination physiology of forest tree species. Recently some researchers reported chemical composition and germination tree species (Marrtinez-Maldonadol et al., 2015; Tonguç, 2019). The aim of the presented preliminary study was to investigate changes in protein and sugar metabolism during the germination and early seedling growth in Turkish red pine.

2. MATERIALS AND METHODS

Red pine seeds were collected in the summer of 2016 and obtained from Sütçüler Forest Management Directory, Isparta. The seeds were stored at 4 °C for 3 months to break dormancy and to promote even germination. Seeds were surface sterilized within 5% sodium hypochlorite for 5 min and washed under tap water to remove residual sodium hypochlorite, and air dried before germination tests.

Germination studies were conducted with using 20 cm petri dishes. Two layers of filter paper, moistened with distilled water, were placed in petri dishes and 30 seeds were placed in each petri dish. Seeds were placed in a germination chamber with alternating day and night lights for 25 days at 20 °C. Germinated pine seeds were removed from the incubator at every 5 days for analysis. Germinating seeds and growing seedlings were dried for 24 h at 50 °C and three replications were used for the analysis of each parameter.

For protein extraction 1 g sample were pulverized with a mortar and pestle in 5 ml cold absolute ethanol and centrifuged at 15000 g for 20 min at 4 °C and supernatant was removed. Pellet was extracted with 5 ml buffer solution (50 mM Tris, 1.2 M NaCl pH 7.0) and incubated on ice for 30 min. After incubation period samples were centrifuged at 15000 g for 20 min at 4 °C and supernatant was collected. Total soluble protein content was determined by Lowry-Hartree method (Hartree, 1972). Different concentrations of bovine serum albumin (0.03-0.15 mg.ml-1) BSA was used to obtain the standard curve, and the absorbance values of the samples were determined at 650 nm with a spectrophotometer.

Extractions of sugars were carried out with 1 g ground sample. 10 ml ethanol (80%) was added onto samples and the samples were incubated at -20 °C overnight and centrifuged at 2000 x g for 5 min. Supernatant was used for the determination total soluble and reducing sugars. Total soluble sugars content was determined with phenol sulfuric acid method (DuBois et al., 1956) and reducing sugars content was determined with Nelson-Somogyi method (Somogyi, 1952). Standard curve was prepared with aqueous solutions of glucose and samples absorbance values were determined at 490 nm and 280 nm for soluble and reducing sugars; respectively.

The data were analyzed with SAS (1998) statistical program. One-way analysis of variance (ANOVA) were performed with 3 replications and Duncan's multiple range test ($p \leq 0.05$) was used to separate the means.

3. RESULTS AND DISCUSSION

Imbibition, germination and seedling growth depends on many physiological processes. Seed storage reserves provide necessary nutrients for the germinating seeds and growing seedlings

until the seed reserves depleted. Mobilization of seed reserves differ among the plants and depends on the type of the reserves seeds accumulate during the seed filling period (Bewley and Black, 1994). The present study investigated protein and sugar metabolism during the 25-day growing period of Turkish red pine.

There were significant differences between germination periods and examined parameters. Soluble protein content was 2.72 mg ml-1 at the beginning and reached the maximum level at day 10 and the minimum value at day 15. At the end of the germination period, its value returned to 2.71 mg ml-1 (Table 1). Protein content changes were presented in Figure 1A.

Total soluble sugars content gradually decreased from 7.48 mg 100 g-1 at day 0 to 5.73 mg 100 g-1 at day 10. After day 10 of germination soluble sugars content started to increase and reached 7.62 mg 100 g-1 at the end of the germination period. Changes in reducing sugars content was similar to soluble sugars content during the experiment. It started to decrease till day 10 (132.0 mg 100 g-1) and it started to increase from day 15 to day 25 and reached 383.37 mg 100 g-1 at day 25. Changes in soluble sugars and reducing sugars contents were presented in Figure 1B and 1C; respectively.

Table 1. Changes during the protein and sugar contents of Turkish red pine during the germination and early seedling growth.

Germination time (day)	Soluble protein content (mg ml^{-1})±SE	Total soluble sugars (mg 100 g^{-1})±SE	Reducing sugars (mg 100 g^{-1})±SE
0	$2.72±0.78^{ab}$	$7.48±0.45^{a}$	$216.63±8.94^{c}$
5	$2.57±0.58^{bc}$	$6.34±0.25^{b}$	$138.63±17.89^{d}$
10	$3.13±0.09^{a}$	$5.73±0.37^{c}$	$132.00±11.21^{d}$
15	$2.21±0.63^{c}$	$7.48±0.54^{a}$	$223.07±21.06^{c}$
20	$2.65±0.60^{abc}$	$7.61±0.58^{a}$	$369.50±16.56^{b}$
25	$2.71±0.80^{ab}$	$7.62±0.67^{a}$	$383.37±8.07^{a}$

*Means followed by the same letters within the columns are not statistically significant from each other at $p≤0.05$.

In this preliminary investigation, we have examined 3 parameters present in red pine seeds and their changes during the germination period. Protein and sugar contents significantly changed during the germination period indicating that they play important roles and their presence might affect germination. Soluble sugars play important roles as energy source and desiccation tolerance during the seed maturation (Koster and Leopold, 1988). Protein content of seeds from different provenances significantly affected seed germination of P. pinaster seeds and protein content ranges between 14.6-19.1 mg g-1 among the populations in Morocco (Wahid and Bounoua, 2013).

There are many reports investigating cone, seed and seedling growth, morphologies and germination behavior of seeds in red pine and other tree species, however; seed chemical composition and its effects on the germination has not been investigated for many tree species including red pine. Research on seed chemical composition of forest trees have been gaining significance recently (Soriano et al., 2011; Tonguç, 2019).

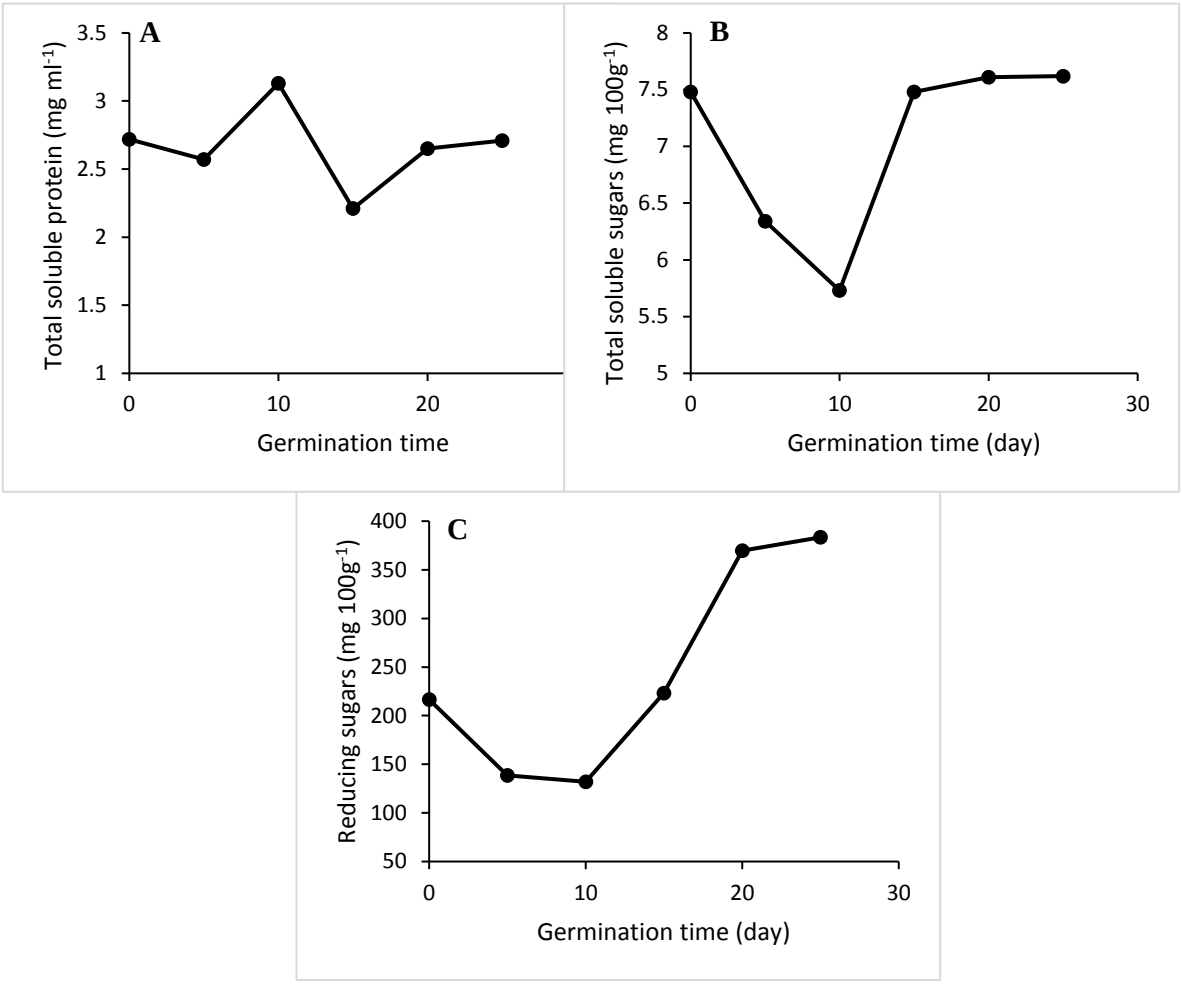

Figure 1A-C. Changes observed during the germination period for protein and sugar contents.

4. CONCLUSIONS AND RECOMMENDATIONS

Protein and sugar contents showed significant differences during the germination and early seedling growth period. Therefore, it will be important to study other primary metabolites and their relationships to germination process in Turkish red pine to better understand germination physiology of this species.

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