SUMMARY

This study was carried out to determine effects of different pretreatment on seed germination and to overcome dormancy in *Acer cappadocicum* seeds. The seeds were collected in 2008 three times with approximately 15-days intervals. In order to overcome dormancy, several germination treatments were applied. The treatments were (1) different seed collection time, (2) soaking in water, (3) cold-moist stratification and (4) GA3 (gibberellic acid) application. The treated seeds were germinated in growing chamber at 5 °C and in greenhouse conditions. This research showed that seeds of *Acer cappadocicum* exhibit physiological dormancy and require stratification period to overcome seed dormancy. The highest germination percentage in the growing chamber subjected to GA3 process after eight weeks of stratification treatment was 62 % for *Acer cappadocicum* seeds. The highest germination percentage in greenhouse was obtained with cold stratification after eight weeks (95 %). It was found out that GA3 treatment had a significant effect on germination in growth chamber + 5 °C but GA3 treatment didn't have a significant effect on germination in greenhouse conditions. GA3 treatment and soaking of unstratified seeds in water for 48 hr didn't have any positive effect on germination value in greenhouse conditions. Although growth chamber and green house results both indicated that seed collection time did not seem to play a role as statistically on seed germination, Duncan's test showed that the third seed collection time was in a different group.

KEY WORDS: *Acer cappadocicum*; seed; dormancy; stratification; gibberellic acid
and Noi 2001). Also, several studies have shown that Acer seeds have dormant embryo (Webb and Wareing 1972; Pinfield et al. 1987; Pinfield and Stutchbury 1990). Pregermination requirements of Acer species seeds vary according to seed ripening time and the nature of dormancy (Phartyal et al. 2002). In Maple species, an endogenous dormancy is generally seen due to requiring a rest period after maturation of the embryo (Ucler and Turna 2005). This occurs in nature during the cold season or during cold stratification period in nursery practice (Pirotto ve Noi 2001). Dormancy was removed by cold-moist stratification in many Acer species (Farmer and Cunningham 1981; Tylkowski 1995; Tremblay et al. 1996; Savage et al. 1998; Macdonald 1999; Gultekin 2007; Farhadi et al. 2013). In addition to cold-moist stratification, gibberellic acid also promotes breaking seed dormancy and stimulates seed germination in many species (Chauhan and Arun 1998). Afterwards, the seeds were harvested at three times with approximately 15-days intervals. Seed collected trees were marked with red oil paint. Thus, the same trees were used for different seed collection times. The harvested seeds were labeled and put into black plastic bags. The seeds were dewinged by hand and air-dried in the laboratory. The seeds were placed in glass jars after they reached approximately dry air humidity (10 ±2 %) and stored in a cooler. The initial moisture content, the 1000-seed weight and seed viability according to Tetrazolium test of Acer cappadocicum seeds were determined for each collection time. The initial moisture content was determined by using drying oven method at 104 ± 1 °C 17 hr (ISTA, 1996).

Although Acer cappadocicum spreads naturally in the Eastern Black sea forests, it can not be produced sufficiently in both private and forest nurseries. The use of naturally spreading species in reforestation studies is one of the main principles. In this study, seed dormancy removal of Acer cappadocicum, one of the important Acer species of the eastern Black Sea region, were studied. The aim of the present study was to investigate the effect of different seed collection time, cold-moist stratification, GA₃ and soaking applications on seed dormancy breaking and germination in Acer cappadocicum seeds.

**MATERIALS AND METHODS**

*Seed material – Sjenenski materijal*

The seeds were collected from Trabzon –Sayrac village (857m, 52°05'65 N, 45°28'759 E, ) in Trabzon, Turkey at three different times with 15-day intervals (on September 12, 2008; on September 27, 2008; on October 10, 2008). The beginning of the greyish brown colour of the seed was decided as first collection time (Chauhan and Arun 1998). Seed collected trees were marked with red oil paint. Thus, the same trees were used for different seed collection times. The harvested seeds were labeled and put into black plastic bags. The seeds were dewinged by hand and air-dried in the laboratory. The seeds were placed in glass jars after they reached approximately dry air humidity (10 ±2 %) and stored in a cooler. The initial moisture content, the 1000-seed weight and seed viability according to Tetrazolium test of Acer cappadocicum seeds were determined for each collection time. The initial moisture content was determined by using drying oven method at 104 ± 1 °C 17 hr (ISTA, 1996).

**Table 1. Laboratory and Greenhouse Experiments**

| Treatments | Laboratory experiments | Greenhouse experiments |
|------------|------------------------|-----------------------|
| Postupci   | Laboratorijski pokus    | Staklenički pokus      |
| 1          | No soaking and direct germination treatment | No soaking and direct sowing treatment |
|            | Bez močenja i direktni postupak klijavosti | Bez močenja i direktni postupak sjetve |
| 2          | 48 hr soaking and germination treatment | 48 hr soaking and sowing |
|            | 48-satni postupak močenja i klijavosti | 48 sati močenja i sjetve |
| 3          | 8 week(w) stratification (st.) and germination treatment | 8 tjedana (t) stratifikacije (st.) i klijanja |

| 4          | No soaking + 50 ppm GA₃ | No soaking + 50 ppm GA₃ |
|            | Bez močenja + 50 ppm GA₃ | Bez močenja + 50 ppm GA₃ |
| 5          | 48 hr soaking + 50 ppm GA₃ | 48 sati močenja + 50 ppm GA₃ |
|            | 48 sati močenja + 50 ppm GA₃ | 48 sati močenja + 50 ppm GA₃ |
| 6          | 8 w stratification (st.) + 50 ppm GA₃ | 8 t stratifikacije (st.) + 50 ppm GA₃ |
|            | 8 t stratifikacije (st.) + 50 ppm GA₃ | 8 t stratifikacije (st.) + 50 ppm GA₃ |
| 7          | No soaking +100 ppm GA₃ | No soaking + 100 ppm GA₃ |
|            | Bez močenja + 100 ppm GA₃ | Bez močenja + 100 ppm GA₃ |
| 8          | 48 hr soaking + 100ppm GA₃ | 48 sati močenja + 100ppm GA₃ |
|            | 48 sati močenja + 100ppm GA₃ | 48 sati močenja + 100ppm GA₃ |
| 9          | 8 w stratification + 100 ppm GA₃ | 8 t stratifikacije + 100 ppm GA₃ |
|            | 8 t stratifikacije + 100 ppm GA₃ | 8 t stratifikacije + 100 ppm GA₃ |
| 10         | No soaking +400ppm GA₃ | No soaking + 100ppm GA₃ |
|            | Bez močenja + 400ppm GA₃ | Bez močenja + 100ppm GA₃ |
| 11         | 48 hr soaking + 400ppm GA₃ | 48 hr soaking + 400ppm GA₃ |
|            | 48 sati močenja + 400ppm GA₃ | 48 sati močenja + 400ppm GA₃ |
| 12         | 8 w stratification + 400 ppm GA₃ | 8 w stratifikacije + 400 ppm GA₃ |
|            | 8 t stratifikacije + 400 ppm GA₃ | 8 t stratifikacije + 400 ppm GA₃ |
Laboratory and Greenhouse experiments – Pokus u laboratoriju i u stakleniku

In this study, treatments of growth chamber and greenhouse are shown below (Table 1).

Laboratory Experiments – Pokus u laboratoriju

The seeds were soaked for 48 hr in water at room temperature (Genc 2007) before germination and sowing treatments in order to break dormancy caused by seed coat. Also, the seeds were treated with cold-moist stratification treatment to break seed dormancy. The seeds were mixed with approximately % 40 humidified sand and placed in plastic bags, and then stored in the refrigerator (at 4°C) for cold-moist stratification treatment (Saatcioglu 1971; Jensen 2001; Yahyaoglu and Olmez 2004). In pre-experiments, the highest germination percentage of stratified seeds was obtained in seeds treated with stratification for 8 weeks. When the stratification period was prolonged, most of the seeds germinated during stratification period. Therefore, stratification period was determined as 8 weeks in this study. As a different treatment, the seeds collected different collection time were treated with GA3 (Giberellic acid; 50, 100, 400 ppm) for 24 hr and germinated in growth chamber (Table 1).

Germination tests were conducted in petri dishes covered with filter paper (ISTA 1996) and 100 (4 X 25) seeds were used for each germination test. Petri dishes and filter paper were sterilized in the oven at 160°C for approximately 2 hours. Also, the seeds were sterilized in a % 2 sodium hypochlorite solution for 10 minute and rinsed in pure water for 5 minute before germination treatments (Jensen 2001). Petri dishes covered and randomly placed in growth chamber. The seeds with radicles longer than 3 mm were tought to be germinated and taken from the petri dishes (Jensen 2001). Germination tests were considered completed when there was no germination for 14 consecutive days (Tremblay et al. 1996). In pre-experiments, the highest germination percentage of Acer cappadocicum seeds was observed in germination experiments at +5°C. Therefore, in this study, the germination experiments were carried out at +5°C.

Greenhouse Experiments – Pokus u stakleniku

In order to evaluate the germination performance of Acer cappadocicum seeds in the greenhouse conditions, seed beds (soil) were used in the greenhouse at East Black Sea Forestry Research Institute. The seeds were subjected to soaking 48 hr water, 8 week stratification, soaking 48 hr water + 8 week stratification and GA3 applications (100 and 400 ppm) (Table 1). 100 seeds were used for each treatment. The seeds were sown on seed bed by using line sowing method (Genc 2007). 50 seeds were sown in each line and sand-forest soil mixture was used as cover material. The irrigation in greenhouse was doneby automatically. The temperature of morning, noon and evening in the greenhouse was recorded regularly from the beginning of germination to the end of germination. Germinants were recorded weekly.

Statistical Analysis – Statistička analiza

In the present study, data were analyzed using the SPSS statistical software. Correlation analysis, Analysis of variance (ANOVA), Duncan’s test and Independent samples t-test were used (Ozdamar 1999).

RESULTS

Laboratory Experiments – Pokus u laboratoriju

The seeds collected at tree different times were germinated in the growing chamber at +5°C after they had been su-
Subjected to soaking 48 hr water, 8 week stratification and GA3 applications (50, 100 and 400 ppm). The difference between the treatments was tested by analyses of variance and the significance of differences between groups was tested by Duncan’s test (Table 2 and Table 3).

There was no effect of seed collection time on germination. The highest germination percentage was recorded after 8 weeks of cold-moist stratification (49.6%). This resulted in increased germination. Soaking in water of nonstratified seeds (stratification 3) wasn’t any significant difference on seed germination (Table 3). GA3 treatment had a significant effect on germination (Table 2) but there wasn’t any difference between GA3 doses (Table 3).

Germination percentages were also evaluated in terms of treatments (Figure 1).

The highest germination percentage in the growing chamber was obtained in seeds collected at second seed collection time and subjected to GA3 process after eight weeks of stratification (62%). Germination percentage was lower in control seeds. The soaking seeds in water for 48 hr wasn’t any significant effect on germination (Figure 1).

**Greenhouse Experiments – *Pokus u stakleniku***

The seeds collected at three different times were sown (15.01.2009) in the greenhouse after they had been subjected to soaking in water for 48 hr, stratification for 8 w and GA3 treatments (100 and 400 ppm). The air temperature in the greenhouse was at +4°C in the morning, +7°C at noon and +9°C in the evening until the last date of germination. The average temperature in the greenhouse was at +11°C in the morning, at +15°C at noon and at 19°C in the evening until the last date of germination. Germinants were counted at weekly. The difference between the treatments was tested by analyses of variance and the significance of differences between groups was tested by Duncan’s test (Table 4 and Table 5).
Although there was no significant difference between seed collection time according to the analysis of variance (Table 4), Duncan’s test showed that the third seed collection time was in a different group (Table 5). This was due to the test’s sensitivity. The effect of stratification was significant on germination (p<0.05). While the average germination percentage of seeds sown after eight weeks of stratification period was 76.11 %, the germination percentage of seeds sown unstratified was 49.16 %. GA3 treatment didn’t have a significant effect on germination. Also, according to the results of the correlation analysis, there was a positive correlation between seed collection time and germination (r=0.442).

Table 4. Results of ANOVA for effects of different pretreatments in the greenhouse

| Variable | Source | Sum of Squares | Degree of Freedom | Mean square | F-value | p-value |
|----------|--------|----------------|-------------------|-------------|---------|---------|
| Collection time | September 12 | 1713.44 | 2 | 856.72 | 2.51 | 0.09 |
| Stratification | September 27 | 13068.05 | 1 | 13068.05 | 38.30 | 0.00* |
| GA3 application | October 10 | 71.44 | 2 | 35.72 | 0.105 | 0.90 |
| Collection time x Stratification | 425.44 | 2 | 212.72 | 0.62 | 0.54 |
| GA3 treatment | 85.22 | 4 | 21.30 | 0.06 | 0.99 |
| Stratification x GA3 application | 4.11 | 2 | 2.05 | 0.06 | 0.99 |
| Collection time x Stratification x GA3 application | 167.88 | 4 | 41.97 | 0.12 | 0.97 |

* p<0.05 (There is a statistically difference)  
* p<0.05 (Postoji statistička razlika)

Table 5. Germination percentages and Duncan’s test groups of Acer cappadocicum seeds

| Variable | Treatments | Mean ± Std. Deviation |
|----------|------------|-----------------------|
| Collection time | September 12 | 59.83 ± 20.29a |
| September 27 | 58.58 ± 21.27a |
| October 10 | 69.50 ± 23.18b |
| *Stratification | 8 week stratification | 76.11 ± 17.79a |
| No stratification | 49.16 ± 16.73b |
| GA3 application | GA3 1:No GA3 | 63.75 ± 23.45a |
| GA3 2:100 ppm GA3 | 61.33 ± 23.72a |
| GA3 3:400 ppm GA3 | 62.83 ± 18.99a |

*The comparison of two independent populations was done by t test to evaluate the effect of stratification (P=0.000)  
*Usporedba dviju neovisnih populacija napravljena je pomoću t testa kako bi se procijenili učinci stratifikacije(P=0.000)
nation percentages were also evaluated in terms of treatments (Figure 2).

The highest germination percentage in the greenhouse was observed in seeds collected at third seed collection time and subjected to eight weeks of stratification period after soaking in water for 48 hr without GA, treatment (95 %). GA, treatment and soaking of unstratified seeds in water for 48 hr didn't have any positive effect on germination value.

DISCUSSIONS
RASPRAVA

In this study, cold-moist stratification period was determined as eight weeks for Acer cappadocicum seeds in the preliminary trials. When the stratification period was prolonged, most of the seeds germinated during stratification period. Yahyaoglu et al. (2006) reported that seed germination during stratification period was an important factor in obtaining low germination in sowing. Because of this reason, stratification process should be continued to species requiring stratification period before germination process until the first germinant appear in stratification medium. Because, the germination of the seeds in stratification medium affected germination percentage negatively. Urgenc (1998) also reported that stratification period could extend from one week to 3-4 weeks or even longer depending on the species. If stratification period extend, the seeds could begin to germinate during stratification medium. This situation was detected in some Maple species (Urgenc 1998; Piotto and Noi 2001; Bonner and Karrfalt 2008). The germination percentage of Acer cappadocicum seeds stratified for eight weeks was higher than seeds unstratified in both the growing chamber and the greenhouse conditions. This result underlines the fact that seeds of Acer cappadocicum exhibit physiological dormancy and require stratification period to overcome seed dormancy. Several researches have already investigated that some maples had seed dormancy and mature Acer seeds require at least eight weeks of cold moist stratification to overcome dormancy (Urgenc 1998; Macdonald 1999; Piotto and Noi 2001; Nasari et al. 2018). However, it is also stated that stratification period should be longer in order to break dormancy in seeds of Acer sacharum (Evans and Blazich 1999), five different Acer species (Yang and Lin 1999), Acer ceasium (Phartyal 2002). Also, Farhadi et al. (2013) pointed out that the highest germination value of Acer velutinum seeds was obtained after 16 weeks of cold-moist stratification. Furthermore, unlike the present study, Yilmaz (2007) reported that the dormancy of Acer trautvetteri seeds was completely removed by three months of chilling but all seed germinated during the chilling period. Therefore, in the present study, stratification period was suggested as eight weeks because of this situation can be cause failure in sown. In this study, it was determined that soaking unstratified seeds in water for 48 hr before germination trial was no statistically significant effect on germination. However, when treatments evaluated on the basis of individual, the highest germination percentage in the greenhouse was obtained from seeds collected at third seed collection time and subjected to eight weeks of stratification period after soaking in water for 48 hr without GA, treatment (95 %) (Figure 2). Similarly, after moist chilling for 16 weeks of seeds after soaking 48 hr in water and germinating at 5:15 °C, germination was 92 % in Acer pensylvanicum (Bourgoin and Simpson 2004). Furthermore, it was observed that soaking different Acer seeds in water for 48 hr before germination trials increased germination rate (Webb and Dumbroff 1969; Webb 1974; Genc 2007 ). According to analysis of variance (Table 2), it was found out that GA, treatment had a significant effect on germination in growth chamber (+ 5 °C) but there wasn’t any difference between GA, doses (Table 3). Similarly, Pawlowski (2009) reported that breaking of dormancy was stimulated by Gibberellic acid in Acer platanoides seeds. GA, treatments of Acer hyrcanum seeds shortened the cold stratification period and increased germination but did not eliminate the requirement of cold stratification of the seeds (Naseri et al. 2018). In this study, it was found out that GA, treatment didn’t have a significant effect on germination in greenhouse conditions. As a result of sowing in the greenhouse, it was observed that GA, treatment was less effective than cold moist stratification treatment for 8 weeks on the germination of the seeds. These results indicate very clearly that GA, treatment of Acer cappadocicum seeds especially stratified before sowing in greenhouse doesn’t have a positive effect on germination. Therefore, GA, application should not preffered in the greenhouse sowing of Acer cappadocicum seeds. Similarly, Stejskalova et al. (2015) found out that in Acer pseudoplatanus gibberellic acid did not increase the germination percentage compared to stratified seeds. Furthermore, Webb and Wareing (1972) reported that GA3 treatments had no effect for breaking dormancy in Acer pseudoplatanus seeds. Although growth chamber and greenhouse results both indicated that seed collection time did not seem to play a role as statistically on seed germination, Duncan’s test showed that the third seed collection time was in a different group (Table 5). The reason for this is the sensitivity of the test. The highest germination (95 %) in the greenhouse was detected in the seeds collected at collection time 3. Also, there was a positive correlation between seed collection time and germination (r=0.442). As a result, the third collection time (in October) should be preffered as seed collection time in Acer cappadocicum seeds, considering that it may vary according to the climatic conditions of the year.

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SAŽETAK

Acer cappadocicum var. cappadocicum široko je rasprostranjen na Kavkazu, u Zapadnoj Aziji i na Himalaji, javlja se u s severoistočnoj Anatoliji u Turskoj na visinama od 400 m do 1600 m, a uobičajen je i u regijama Ordu, Giresun, Trabzon, Rize i Artvin u Turskoj. Iako se Acer cappadocicum prirodno širi u šumama istočnog Crnog mora, on se ne može proizvesti u dovoljnim količinama u privatnim i šumskim rasadnicima. U istraživanju poželjno je jedno od glavnih naslova otklanjanje dormantnosti sjemenaka Acer cappadocicum, jedne od važnih vrsta Acer u području istočnog Crnog mora. Ovaj rad bavi se istraživanjem utjecaja na ovom području istraživanje, a značajniji je utjecaj predtretmana na klijavost sjemenaka i otklanjanja dormantnosti sjemenaka Acer cappadocicum. Sjeme je prikupljeno 2008. godine u tri navrata u intervalima od približno 15 dana. Kako bi se otklonila dormantnost, primijenjeno je nekoliko tretmana. Tretmani su uključivali (1) različito vrijeme prikupljanja sjemenaka, (2) potapanje u vodi, (3) hladno-vlažnu stratifikaciju i (4) primjenu GA3 (giberelinska kiselina). Tretirano sjeme podvrgnuto je klijavnom korištenju u stakleničkim uvjetima. Ovim istraživanjem je utvrđeno da sjeme Acer cappadocicum pokazuje fiziološku dormantnost i da je to otklanjanje dormantnosti sjemenaka potrebno razdoblje stratifikacije. U preliminarnim pokusima, sjeme Acer cappadocicum podvrgnuto je hladno-vlažnoj stratifikaciji tijekom osam tjedana. Kad je period stratifikacije prošao, većina sjemenaka prokljila je tijekom perioda stratifikacije. Najviši postotak klijavnosti u komori rasta izloženom postupku s GA3 nakon osam tjedana stratifikacije iznosi je 62%. Najviši procenat klijavnosti u stakleniku postignut je postupkom hladne stratifikacije nakon osam tjedana (95%). Utvrđeno je da tretman s GA3 nije imao značajniji utjecaj na klijavost u stakleničkim uvjetima. Prema tomu, za sijanje sjemenaka Acer cappadocicum u stakleničkim uvjetima ne preporučuje se primjena GA3. Tretman s GA3 i potapanje nestriječiranog sjemenaka u vodi 48 sati nije imao pozitivne učinke na vrijednosti klijavnosti u stakleničkim uvjetima. Iako rezultati dobiveni u komori rasta i stakleniku pokazuju da vrijeme prikupljanja sjemenaka nije statistički utjecalo na klijavost sjemenaka, Danačkov test ukazuje na to da se klijavost sjemenaka sa kupljenog u trećem navratu signifikantno razlikuje u odnosu na klijavost sjemenaka sa kupljenog u prva dva navrata. Rezultati pokazuju da je najbolje vrijeme za prikupljanje sjemenaka Acer cappadocicum ono iz trećeg navrata (u listopadu), ali ono može i varirati ovisno o klimatskim uvjetima tijekom godine.

KLJUČNE RIJEČI: Acer cappadocicum; sjeme; dormantnost; stratifikacija; giberelinska kiselina