In this study, seed dormancy removal and germination characteristics of *Acer trautvetteri* Medvedev, is one of the major maple species of the eastern Black Sea region, were studied. To do that, the seeds were collected in 2008 three times with approximately 15-days intervals. In order to remove 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 indicated that seeds of *Acer trautvetteri* exhibit physiological dormancy and require stratification period to remove seed dormancy. The highest germination percentage in the growing chamber subjected to GA3 application after eight weeks of stratification treatment was 66 % for *Acer trautvetteri* seeds. The highest germination percentage in greenhouse was obtained with cold stratification after eight weeks (81 %). Although GA3 treatment had a positive effect on germination in growth chamber + 5 °C, GA3 treatment had a negative effect on germination in greenhouse conditions. Soaking in water of nonstratified seeds wasn't any significant difference on seed germination. There was a positive correlation between seed collection time and germination (r=0.59). As a result, the third collection time (in October) should be preferred as seed collection time in *Acer trautvetteri* seeds, considering that it may vary according to the climatic conditions of the year.

**KEY WORDS:** *Acer trautvetteri*; seed; dormancy; stratification; gibberellic acid

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**INTRODUCTION**

*Acer trautvetteri* Medvedev has a geographic distribution in Caucasia and Turkey. It ranges between 400 m and 2100 m. It is especially common in North Eastern Anatolia and is also common in the region of Kırklareli, Balıkesir, İstanbul, Kocaeli, Bolu, Sinop, Ordu, Giresun, Trabzon, Rize and Artvin in Turkey (Ansin and Ozkan 1997; Davis 1969). Maple are well known for their autumnal colour (Kumar et al. 2017). Also, many of the maples have ornamental value because of their attractive foliage, interesting crown shape, flowers and fruit (Ansin and Ozkan 1997; Zasada and Strong 2008; Ognjenović et al. 2018). Maple wood has a light color and is used in veneer and furniture manufacturing (Ulker and Hiziroglu 2018). *Acer trautvetteri* grows fast and has an economic value for the forest industry (Korkut and Büyüksari 2006). Furthermore, *Acer trautvetteri* wood could be used by applying proper heat treatment methods in some areas such as windows frame (Korkut and Guller 2008). *Acer* seeds are accepted by several researchers in the class of seeds with seed dormancy (Bradbeer 1988; Peroni 1995;
Erdogan Genc and Ucler 2020). In addition to cold-moist stratification, gibberellic acid also promotes breaking seed dormancy and stimulates seed germination in many species (Chen and Chang 1972; Beyhan et al. 1999; Phartyal et al. 2002). Although Acer trautvetteri 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 trautvetteri, 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 removal and germination in Acer trautvetteri seeds.

**MATERIALS AND METHODS**

**MATERIJALI I METODE**

**Seed material – Sjenenski materijal**

The seeds were collected from district of Şalpazarı, Geyikli village ( 800m, 53 20 50 N, 45 16 450 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). 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 (+4 °C). The initial moisture content, the 1000-seed weight and seed viability according to Tetrazolium test of Acer trautvetteri seeds were determined for each collection time. The initial moisture content was determined by using drying oven method at 104 ± 1 °C 17 hour (ISTA, 1996).

| Treatments | Laboratory experiments | Greenhouse experiments |
|------------|------------------------|------------------------|
| 1          | No soaking and direct germination treatment | No soaking and direct sowing treatment |
|            | Bez močenja i sjetva odmah nakon sakupljanja | Bez močenja i sjetva odmah nakon sakupljanja |
| 2          | 48 hr soaking and germination treatment | 48 sati močenja i sjetva |
| 3          | 8 week (w) stratification (st.) and germination treatment | 48 hr soaking + 8 w stratification |
| 4          | No soaking + 50 ppm GA3 | 48 sati močenja i sjetva |
| 5          | 48 hr soaking + 50 ppm GA3 | Bez močenja + 100 ppm GA3 |
| 6          | 8 w stratification (st.) + 50 ppm GA3 | 48 h soaking + 100ppm GA3 |
| 7          | 8 t stratifikacije (st.) + 50 ppm GA3 | 48 sati močenja + 100ppm GA3 |
| 8          | No soaking + 100 ppm GA3 | No soaking + 8w stratifikacije + 100ppm GA3 |
| 9          | Bez močenja + 100 ppm GA3 | Bez močenja + 8 t stratifikacije + 100ppm GA3 |
| 10         | 48 hr soaking + 100ppm GA3 | 48 hr soaking + 8 w stratifikacije + 100ppm GA3 |
| 11         | 48 sati močenja + 100ppm GA3 | 48 sati močenja + 8 t stratifikacije + 100ppm GA3 |
| 12         | No soaking + 400 ppm GA3 | No soaking + 8w stratifikacije + 400ppm GA3 |
|            | Bez močenja + 400 ppm GA3 | Bez močenja + 8 t stratifikacije + 400ppm GA3 |
|            | 48 hr soaking + 400ppm GA3 | 48 hr soaking + 8 w stratifikacije + 400 ppm GA3 |
|            | 48 sati močenja + 400 ppm GA3 | 48 sati močenja + 8 t stratifikacije + 400 ppm GA3 |
|            | 8 w stratifikacije + 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 – Tretmani u laboratoriju i u stakleniku

In this study, treatments of growth chamber and greenhouse are shown in Table 1.

Laboratory Experiments – Istraživanje u laboratoriju

The seeds were soaked for 48 hour in water at room temperature (22°C ± 2°C) (Genc 2012) 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; Yahyaoglu and Olmez 2005). In pre-experiments, the highest germination percentage of stratified seeds was obtained in seeds treated with stratification for 8 weeks. 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 hours and germinated in growth chamber (Table 1).

Germination tests were conducted in petri dishes (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. Petri dishes covered and randomly placed in growth chamber. The seeds with radicles longer than 3 mm were thought 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 trautvetteri seeds was observed in germination experiments at +5°C. Therefore, in this study, the germination experiments were carried out at +5°C.

Greenhouse Experiments – Istraživanja u stakleniku

In order to evaluate the germination performance of Acer trautvetteri 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 hours water, 8 week stratification, soaking 48 hours 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 2012). 50 seeds were sown in each line and sandforest soil mixture was used as cover material. The irrigation in greenhouse was done by automatically. The temperature of morning, noon and evening in the greenhouse was measured by thermometer and 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 2010).

RESULTS

Laboratory Experiments – Istraživanja u laboratoriju

The seeds collected at three different times were germinated in the growing chamber at +5°C after they had been subjected to soaking 48 hours 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)

| Variable Source | Sum of Squares | Degree of freedom | Mean square | F-value | p-value |
|-----------------|----------------|------------------|-------------|---------|---------|
| Collection time | 308,22         | 2                | 154,11      | 0,5     | 0,60    |
| Vrijeme sakupljanja | 11160,22 | 2                | 5580,11     | 18,32   | 0,00*   |
| Stratification  | 8868,00        | 3                | 2956,00     | 9,70    | 0,00*   |
| GA3 application | 714,00         | 6                | 119,00      | 0,39    | 0,88    |
| Primjena GA3  | 1459,33        | 6                | 243,22      | 0,79    | 0,57    |
| Collection time x Stratification | 2944,44 | 4 | 736,11 | 2,41 | 0,05    |
| Vrijeme sakupljanja x Stratifikacija | 714,00 | 6 | 119,00 | 0,39 | 0,88    |
| Collection time x GA3 application | 1062,66 | 12 | 88,55 | 0,29 | 0,99    |
| Vrijeme sakupljanja x primjena GA3 | 1591,33 | 6 | 243,22 | 0,79 | 0,57    |

*p< 0,05 (There is a statistically difference)

* "p< 0,05 (Postoji statistička razlika)"
There was no effect of seed collection time on germination. Stratification treatment had a significant effect on germination (p<0.05). There were no significant interactions between treatments (Table 2). The highest germination percentage was recorded after 8 weeks of cold-moist stratification (52.25%). This result showed that 8 weeks cold-moist stratification significantly increased germination. Soaking in water of nonstratified seeds 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 first seed collection time and subjected to 400 ppm GA₃ application after eight weeks of stratification (66%). Germination percentage was lower in control seeds. The soaking seeds in water for 48 hour wasn't any significant effect on germination (Figure 1).

### Table 3. Germination percentages and Duncan's test groups

| Variable Source | Treatments | N      | Mean value ± St. Deviation |
|----------------|------------|--------|---------------------------|
| Collection time | September 12 12. rujna | 48     | 42.25 ± 24.36 a           |
|                | September 27 27. rujna | 48     | 39.83 ± 20.49 a           |
|                | October 10 10. listopada | 48     | 38.75 ± 15.61 a           |
| Stratification* | 8 week stratification | 48     | 52.25 ± 20.61 a           |
| * Stratification | No stratification | 48     | 37.25 ± 19.24 b           |
|                | 48 hour soaking water + no stratification | 48 | 31.33 ± 19.19 b |
| GA₃ application | GA₃ 1: No GA₃ | 36     | 27.44 ± 15.91 a           |
|                | GA₃ 2: 50 ppm GA₃ | 36     | 40.44 ± 19.23 b           |
|                | GA₃ 3: 100 ppm GA₃ | 36     | 45.78 ± 19.60 b           |
|                | GA₃ 4: 400 ppm GA₃ | 36     | 47.44 ± 20.88 b           |

*Pretreatments of stratification and soaking + no stratification were evaluated under this variable name
* Predtretmani stratifikacije i močenja + bez stratifikacije procijenjeni su pod ovim varijabilnim imenom

![Figure 1](image1.png)

**Figure 1.** The effect of seed collection time, soaking in water, stratification and GA₃ treatments on germination

**Slika 1.** Utjecaj vremena prikupljanja i močenja u vodi, stratifikacije i primjene GA₃ na kljavost sjemena
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 hours, stratification for 8 weeks 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 at the beginning of first germination (26.01.2009). 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).

There was no effect of seed collection time on germination in the greenhouse. The effect of stratification was significant on germination (p<0.05) (Table 4). While the average germination percentage of seeds sown after eight weeks of stratification period was 53.55 %, the germination percentage of seeds sown unstratified was 38.72 % (Table 5). GA₃ treatment had a significant effect on germination but this effect was negative. There was a significant interaction between seed collection time and stratification (Table 4).

The highest germination was obtained in seeds collected at third collection time (October 10) and sown after 8 week cold stratification (60.16 %). Also, according to the results of the correlation analysis, there was a positive correlation between seed collection time and germination (r=0.59). Germination 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 hour without GA$_3$ treatment (81%).

**DISCUSSIONS**

The germination percentage of *Acer trautvetteri* seeds stratified for eight weeks was higher than seeds unstratified in both the growing chamber and the greenhouse conditions. This fact confirms that seeds of *Acer trautvetteri* exhibit physiological dormancy and require stratification period to remove seed dormancy. Similarly, Yılmaz (2007) and Varbeva and Iliev (2015) reported that *Acer trautvetteri* seeds exhibit physiological 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; Piotto and Noi 2003; Nasari et al. 2018; Erdogan Genc and Ucler 2020). Also, Kumar et al. (2017) obtained highest percentage of germination in *Acer acuminatum* after cold moist stratification for a period of 60 day. Whereas, 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. In the present study, cold-moist stratification period was determined as eight weeks for *Acer trautvetteri* seeds in the preliminary trials. When the stratification period was prolonged, most of the seeds germinated during stratification period. This situation was also detected in some *Maple* species (Urgenc 1998; Piotto and Noi 2003; Varbeva and Iliev 2015). Therefore, in the present study, stratification period was suggested as eight weeks because of this situation can be cause failure in sown. However, it is also stated that stratification period should be longer in order to break dormancy in seeds of *Acer saccharum* (Evans and Blazich 1999), five different *Acer* species (Yang and Lin 1999), *Acer ceasium* (Phartyal et al. 2003). 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. In this study, it was determined that soaking unstratified seeds in water for 48 hours before germination trial was no statistically significant effect on germination in growth chamber conditions (Table 2). 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 hours without GA$_3$ treatment (81%) (Figure 2). Similarly, Bourgoin and Simpson (2004) reported that the highest germination value of *Acer pensylvanicum* seeds was obtained from seeds subjected to the moist chilling for 16 weeks after soaking 48 hours (92%). Furthermore, it was observed that soaking different *Acer* seeds in water for 48 hr before germination trials increased germination rate

![Figure 2. The effect of different pretreatments on germination of Acer trautvetteri seeds in greenhouse](image-url)
In this study, it was found out that GA treatment of cold stratification of the seeds (Naseri et al. 2018) increased germination but did not eliminate the requirement of cold stratification of the seeds. In this study, it was found out that GA treatment had a significant effect on germination in greenhouse conditions (Table 4), but this effect was a negative. These results indicate very clearly that GA treatment of Acer trautvetteri seeds especially stratified before sowing in greenhouse doesn’t have a positive effect on germination. Therefore, GA application should not be preferred in the greenhouse sowing of Acer trautvetteri seeds. The same result has been determined in Acer cappadocicum seeds (Erdogan Genc and Ucler 2020). Similarly, Stejskalová et al. (2015) found out that gibberellic acid did not increase the germination percentage compared to stratified seeds in Acer pseudoplatanus. Furthermore, Webb and Wareing (1972) reported that GA3 treatments had no effect for breaking dormancy in Acer pseudoplatanus seeds. Whereas, Zare (2014) obtained the highest percentage of germination in Acer menziesii subsp. turcomanicum after 500 ppm and 250 ppm gibberellic acid treatments with five months moist chilling. Although growth chamber and greenhouse results both indicated that seed collection time did not seem to play a role as statistically on seed germination, there was a significant interaction between seed collection time and stratification in greenhouse conditions (Table 4). The highest germination was obtained in seeds collected at third collection time and sown after 8 weeks cold stratification (60.16 %). In addition, when germination percentages were also evaluated in terms of treatments, the highest germination (81 %) 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=0.59). As a result, the third collection time (in October) should be preferred as seed collection time in Acer trautvetteri seeds, considering that it may vary according to the climatic conditions of the year and different location on area of natural distribution of this species or population.

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SAŽETAK

Vrsta Acer trautvetteri Medvedev geografski je rasprostranjen na Kavkazu i u Turskoj. Pridolazi na visinama od 400 do 2100 m. U ovom radu raspravljamo o savladavanju dormantnosti i značajkama klijanja sjemena vrste Acer trautvetteri Medvedev, jedne od glavnih vrsta javora u istočnom crnornoskom području. U tu je svrhu godine 2008. prikupljano sjeme u tri navrata s razmakom od 15 dana. Kako bismo savladali dormantnost, primijenili smo nekoliko tretmana klijanja. Tretmani su uključivali (1) različito vrijeme sakupljanja sjemena, (2) namakanje u vodi, (3) hladno-vlažnu stratifikaciju i (4), primjenu GA₃ (giberelinske kiseline). Tretirano sjeme klijalo je u komori rasta pri 5°C i u stakleničkim uvjetima. Ovom studijom utvrđeno je da sjeme vrste Acer trautvetteri pokazuje fiziološku dormantnost i da je za savladavanje dormantnosti potreban period stratifikacije. Najviši postotak klijavosti sjemena vrste Acer trautvetteri u komori rasta uz primjenu GA₃ nakon osam tjedana stratifikacije iznosio je 66%. Najviši postotak klijanja u stakleniku postignut je hladnom stratifikacijom nakon osam tjedana (81%). Postotak klijanja sjemena vrste Acer trautvetteri stratificiranog tijekom osam tjedana bio je viši od postotka klijanja nestratificiranog sjemena i u komori rasta i u stakleničkim uvjetima. Ova činjenica potvrđuje da sjeme vrste Acer trautvetteri pokazuje fiziološku dormantnost i da je za savladavanje dormantnosti sjemena potreban period stratifikacije. Iako je tretman s GA₃ imao pozitivan utjecaj na klijavost u komori rasta pri +5°C, tretman s GA₃ negativno je utjecao na klijanje u stakleničkim uvjetima. Namakanje nestratificiranog sjemena u vodi nije pokazalo neku značajniju razliku na klijavost sjemena. Postoji pozitivna korelacija između vremena prikupljanja sjemena i klijanja (r=0.59). Shodno tome, preporučuje se treće vrijeme prikupljanja (u listopadu) sjemena vrste Acer trautvetteri. Točno vrijeme sakupljanja sjemena može varirati ovisno o klimatskim godišnjim uvjetima.

KLJUČNE RIJEČI: Acer trautvetteri; sjeme, dormantnost, stratifikacija, giberelinska kiselina.