JEB
Website : www.jeb.co.in
E-mail : editor@jeb.co.in

Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

The effects of selected pre-treatments on germination of seeds of Oriental hornbeam (*Carpinus orientalis*)

Halil Barış Özel*

Department of Silviculture, Faculty of Forestry, University of Bartin, Bartin-74100, Turkey

*Corresponding Author E-mail: **halilbarisozel@yahoo.com**

Publication Info

Paper received:
13 September 2014

Revised received:
16 March 2015

Re-revised received:
13 April 2015

Accepted:
28 April 2015

Abstract

In the present study, the effect of some pre-treatments implemented on seeds of Oriental hornbeam (*Carpinus orientalis*), which has wide geographical variation along Turkey on germination percentage values were investigated. For this purpose, 13 different pre-treatments were implemented to seeds obtained from 17 different populations. According to the obtained results (except control seeds), pre-treatments leading to lowest germination percentage value (8.1%) in Oriental hornbeam seeds was PT10: Keeping seeds for 90 min in sulfuric acid, while highest germination percentage (86.58%) has been obtained with pre-treatment PT13: Implementation of 40% dose of Baikal EM1 + Biohoumous mixture to the seeds, while lowest germination percentage (40.50%) was observed on seeds collected from P7 (Bartın-Kozcağız) population, highest germination percentage was observed in seeds obtained from P17 (Artvin-Hopa) population.

Key words

Germination percentage, Oriental hornbeam, Pre-treatment

Introduction

Forest degradation negatively affects the characteristics of forest thereby reducing the value and production of its goods and services. This change is caused due to disturbances (although not all disturbance causes degradation), which may vary in extent, severity, quality, origin and frequency. Disturbance may be natural (e.g. that caused by fire, storm or drought), human-induced (e.g. through harvesting, road construction, shifting cultivation, hunting or grazing) or due to combination of both. Human-induced disturbance may be intentional (direct) such as logging or grazing, or it may be unintentional (indirect) like spread of an invasive alien species (FAO, 2014). On the other hand, increase in raw materials and services obtained from these forests were 68.4% in 2014. Despite this increase, decrease in productivity of the forests throughout the world has reached to 58.6%. Particularly, due to biotic and abiotic factors such as overuses of fuel wood incorrect silvicultural approaches, failed foresting activities, opening activities, fires, irregular structuring, insects, bacteria, fungi, snow and storm, the forests loss level has decreased by 78.3% in Africa, 32.6% in Europe and 56.3% in central and eastern Asia (FAO, 2014). Depending on ecological factors diversity, Turkey is a rich country from the aspect of forest wealth and bio-diversity.

Turkey has approximately 22.1 million ha forest area, and this equals to approximately 27.5% of country's total surface area. 52% of this area is productive, and the rest 48% is unproductive area (Anonymous, 2012). The ecological requirements of these unproductive and ruined forest lands is appropriate for growing broad-leaved species rather than needle-leaved species, and transforming these lands into productive form again will be faster via establishing broad-leaved tree forests. In parallel with increasing requirement of wood of broad-leaved trees throughout the world has increased interest on broad-leaved trees and their forests gradually. Improvement of forest trees aims at growing forests having quality and development potential suitable for economy's requirement by utilizing inherited characteristic and variation of forest trees. An important function of tree improvement is to find, to grow, and to propagate productive individuals having some superior characteristic among the natural generations. As genetic source in tree improvement, firstly the natural stands (natural populations) are considered, and the most suitable population for the desired product and the region where the desired product will be produced is chosen among them (Assis and Resende, 2011).

One of the most necessary steps is the production of seeds because many plants are fed their seeds and regular and redundant seed production is desired for the process (Tunçtaner, 2007). The success of foresting via planting or sowing depends on the origin of seeds and their genetic characteristics (Yahyaoğlu and Ölmez, 2005; Üçler and Turna, 2006).

From this aspect, the forest growing activities require enough information about collecting seeds of known origin and information about germination of these seeds. Storage of collected seeds affects germination skills of seed. For this reason, storing collected seeds for long duration is of great importance. *Ex-situ* storage of plants is gaining importance day by day. One of the *ex-situ* protection method is to preserve seeds for long duration without degrading their quality. In hornbeam seeds, there is germination limitation rising from embryo. As embryos in hornbeam seeds are not well-developed, they can stay in seedbed and can continue germinating next year. In view of the above, the present study aimed to determine seed characteristics of Oriental hornbeam (*Carpinus orientalis*), which is one of two natural *Carpinus* species in Turkey, and to obtain highest germination percentage values by eliminating germination obstacles *via* various pre-treatments.

Materials and Method

The seeds of hornbeam used in the present study was collected from 17 populations (P1-P17) from Western and Eastern Black Sea region (Fig. 1) with contributions of Directorate of Forest Trees and Seeds Improvement Researches.

Seeds were collected in late September and early October 2014 which was the most appropriate ripening period of Oriental hornbeam (*Carpinus orientalis*). They were collected through complete field scanning at 135 to 1266 m altitude in a way representing 3 different altitude belts.

During seed collection, attention was paid to naturally of selected population, and selection of population among non-treated stands or stands that were treated very few,

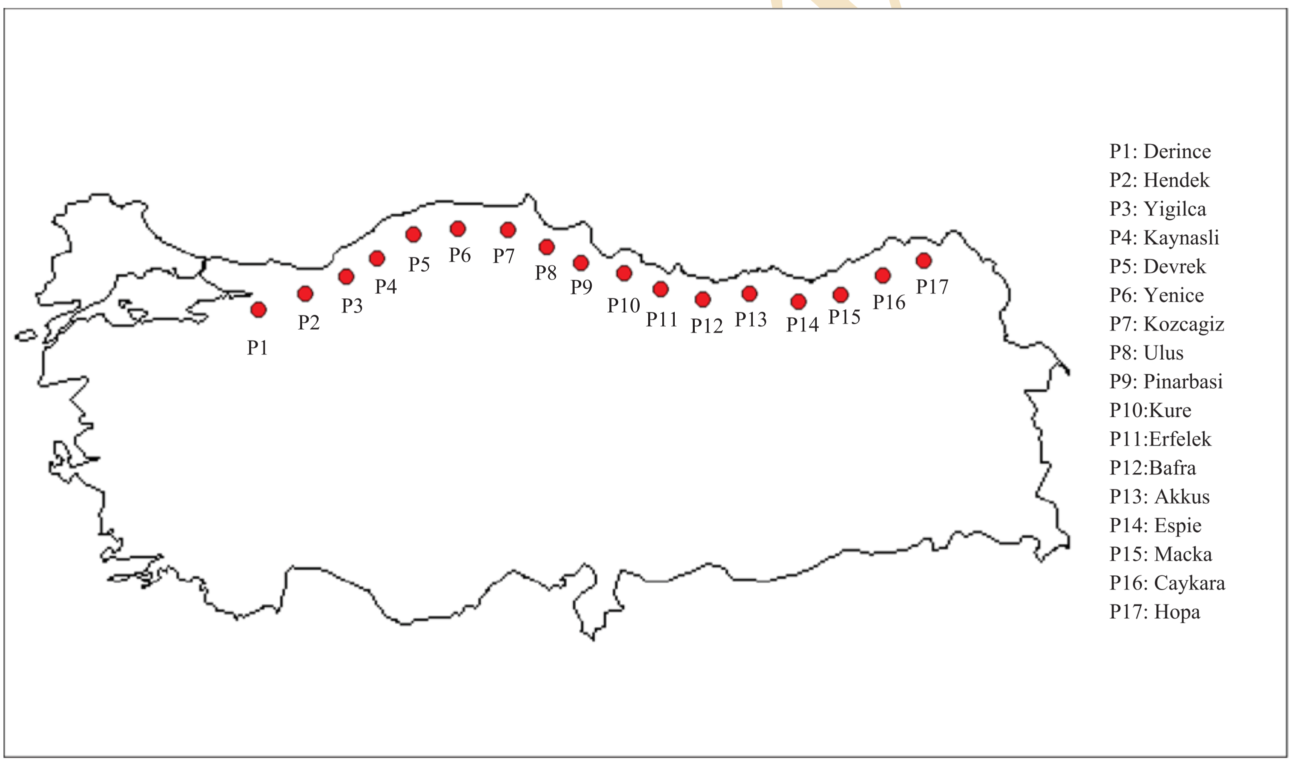

Fig. 1 : Geographical locations of populations (P1-P17) where the seeds have been collected

especially the ones having normal or almost-normal closure. While selecting trees within the population, during sampling, attention was paid to keep 150 m distance between sample trees for minimizing the affinity possibility and to ensure best representation of the population. Seeds collected were put into different bags and labeled. After collection, seeds were taken to laboratory for further investigations. Seeds were separated from fruit cover by hand and then observed for disease, color, damage and undeveloped seeds were removed. Clean seeds were then put into sealed nylon bags and labeled for each population and tree.

After seeds were removed from dried fruits, initial weight of per 1000 seeds of oriental hornbeam was calculated. For this purpose, by counting seeds of each of the trees in sample area separately, 8x100 seeds randomly chosen from 43 trees were calculated, and then weighed in precision balance. By placing the values obtained from weighing process in the formula, the weight of per 1000 seeds was calculated.

In order to determine the fullness rate of seeds, seeds obtained from 3 different populations, a total of 12.900 seeds (300 (3x100) per tree) were utilized. For revealing full and consequently healthy seeds, floatation method was used with 90% ethanol solution (Güney *et al.*, 2014).

In order to remove germination inhibition rising from seed boll and embryo in oriental hornbeam seeds, some pre-treatments were given which are as follows: PT1-Control (floatation in alcohol); PT2-30 days of cold folding; PT3-30 days of cold folding + gibberellic acid (700 ppm); PT4-60 days of cold folding; PT5-60 days of cold folding + gibberellic acid (700 ppm); PT6-90 days of cold folding; PT7-90 days of cold folding + gibberellic acid (700 ppm); PT8-800 ppm gibberellic acid (GA3) treatment on seeds; PT9-10,000 ppm citric acid ($C_6H_8O_7$) treatment on seeds; PT10-Keeping seeds in sulfuric acid for 90 min.; PT11-40% doze of Baikal EM1 treatment on seeds; PT12-40% dose of biohoumous treatment on seeds and PT13-40% doze of Baikal EM1 + biohoumous mixture treatment on seeds.

The pre-treatments mentioned above were implemented under open-air conditions, and a total of 13 different treatments were implemented. All the treatments were implemented on seeds obtained from the method of floatation in alcohol. Initially, in order to remove germination obstacle of oriental hornbeam species, seeds were taken into cold folding to determine the effects of storage duration which were determined to be 30, 60 and 90 days, 3x70 pcs seeds for each folding duration was placed into polyethylene bags containing sand, 40% moisture, and then kept at +4ºC for folding. After seed samples were placed in polyethylene bags, their population was written on the labels on the bags.

After pre-treatments made on seeds, they were labeled according to population numbers, were kept in sealed bags and made ready for planting. In order to perform planting, 2 seedbeds were prepared in greenhouse of Bartın University, Faculty of Forestry. The seedbeds were prepared with sand-forest soil mixture. For planting seeds, line planting method was followed, and planting was executed according to random try pattern. In seedbeds prepared, lines having depth 3 folds of seed's height was opened with a stick. 3x70 seeds were planted with samples. Without applying any pre-treatment and with pre-treatment implementation, seeds were planted into 2 lines for each tree and population. In the present study, 13 different pre-treatments were implemented for each of the altitude zone, a total of 4620 seeds were planted for these pre-treatments. Approximately, 10 trees were used from each altitude zone, 9870 seeds were planted in tree-based planting performed for 170 trees. A total of 210 lines were used for all the planting operations.

As covering material, sand-forest soil mixture was used. Labels indicating the name of population and list of pre-treatment implemented were placed at beginning of the line, and seedbeds were protected against external factors by covering them with cages. Watering in open-air was done regularly and weeds in seedbeds were cleaned at regular intervals. Seed germination was counted from the beginning and recorded weekly.

In order to assess the data, statistical analyses were made on data obtained from measured characters by SPSS package software. In order to determine whether or not each of characters showed variation among the population or within the population depending on altitude, one-way and two-way analyses of variance (99%significantly) and Duncan test (95% significantly) were performed (Özdamar, 1999; Ercan, 1997).

Results and Discussion

In order to remove germination obstacle and to determine difference between germination percentage of the population, 13 different pre-treatments were given, and germination was noted.

Table 1 shows that highest germination percentage values were noted PT13 pre-treatment was implemented in P17, P16 and P14 population. The lowest germination percentage values were noted in P7 and P8 population with PT10. Merou *et al.* (2012) on *Carpinus orientalis* Mill., 1, 2, and 3 months of cold folding at 5ºC has been implemented to mechanically injured seeds after cold folding for 1, 2, and 3 months at 5ºC and application of 15 and 30 min. of H_2SO_4 treatment in order to remove the germination obstacle of the seeds.

Table 1 : Germination percentage results of populations where the different pre-treatments have been implemented

Pre-treatments	Germination percentage values (%)																
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**	**P13**	**P14**	**P15**	**P16**	**P17**
PT1	8.3	7.5	7.2	8.3	7.0	6.5	5.4	6.8	5.5	5.2	7.4	8.3	8.0	7.6	6.8	5.4	5.8
PT2	21.8	20.4	22.5	23.6	20.4	21.8	19.7	20.3	19.2	18.7	21.5	22.8	22.6	21.7	20.3	20.2	21.6
PT3	14.9	17.6	17.3	15.4	15.0	14.8	14.2	15.5	15.4	14.8	14.7	18.2	18.0	17.6	16.8	16.3	17.2
PT4	16.5	18.3	18.8	17.6	17.2	15.3	16.4	17.6	17.3	16.7	16.9	21.0	19.5	18.7	18.2	18.3	18.5
PT5	23.7	24.5	25.6	24.8	25.2	23.4	24.2	26.3	25.7	24.8	25.4	26.8	26.4	25.6	25.1	25.4	25.8
PT6	13.3	15.2	14.8	14.6	14.3	12.5	12.8	13.5	13.6	14.5	14.8	16.4	16.3	16.0	16.2	16.4	16.3
PT7	11.5	12.3	10.5	11.6	10.4	10.6	11.2	10.8	10.7	11.4	12.6	13.1	13.0	13.2	12.7	12.5	12.4
PT8	24.3	24.2	23.6	24.5	24.3	21.5	22.6	21.7	21.8	24.5	25.4	26.7	26.9	27.5	26.8	26.4	26.5
PT9	35.3	35.6	35.4	35.2	32.3	24.5	26.8	25.7	33.6	37.4	38.2	39.3	40.1	38.7	40.0	41.6	42.4
PT10	8.5	8.2	8.3	7.6	7.4	7.3	6.8	6.5	8.1	8.3	8.8	8.2	8.5	8.3	8.4	8.2	8.6
PT11	72.4	73.5	73.8	73.6	68.8	68.6	56.8	52.4	72.6	75.6	75.8	74.7	75.5	72.4	76.4	73.5	75.4
PT12	67.6	65.4	65.3	64.2	61.6	65.2	60.4	63.7	65.8	67.8	67.4	68.5	68.2	69.3	68.2	68.4	69.3
PT13	83.2	83.6	84.2	78.6	78.4	85.4	74.2	84.6	84.3	88.2	89.5	91.2	92.4	93.4	92.5	93.6	94.5

As a result, the germination percentage reached almost 100% with in 3 months of cold folding, but it was found that germination percentage was less in seeds after chemical and mechanical treatment. For this reason, at the end of the study, it was observed that mechanically injuring the seeds led to lethal effect on seeds and chemical treatments might have damaged the embryo. Czapracki and Holubowicz (2010), in their study on *Carpinus betulus*, applied hot-cold folding operations at different durations in order for planting operations to be successful and in order to break germination obstacle. In the present study, where 5 different pre-treatments were implemented in order to remove germination obstacle, most appropriate folding duration was determined. As a result, it was found that the most appropriate medium was hot folding in turf and sand for 4 weeks at 20-22°C and cold folding for 16 weeks at 3-5°C and 0.5% concentration of GA3 decreased the folding duration. In an another study on *Carpinus orientalis* and *Carpinus betulus* species, hot folding, cold folding and GA3 treatments was given in order to remove germination obstacles of both the two species. Firstly, the seeds were taken under hot folder for 1 or 2 months at 20-25ºC, and then under cold folding at 3-5 ºC for 0, 1, 2, 3, and 4 months for initial germination experiment and then, after treatment with 500, 1000 and 2000 ppm GA3 the seeds were under cold folding at 3-5 ºC for 0, 1, 2, 3, and 4 months. The results revealed that the highest germination percentage value was obtained from *Carpinus orientalis* with 2000 ppm GA3+ 4 months cold folding (90.8%), while highest germination in *Carpinus betulus* species was observed with 1000 ppm GA3+ 4 months cold folding (89.2%) treatment (Pipinis *et al.,* 2012). In an another study on *Carpinus loxiflora*, *Carpinus tshonoskii* and *Carpinus cordata* species, germination obstacle 3 species, and the effects of cold folding application on germination was determined. After cold folding application to seeds, it was determined that the germination percentage exceeded 80% in *C. laxiflora* and *C. tshonoskii*, but seeds of *C. cordata* could germinate only after being kept in cold folding for 10 months, and couldn't germinate in darkness (Suzuki, 2000). In a similar research, highest germination percentage of pretreatments were found with citric acid (5000) in population 1, GA3 (500 ppm) in population 2, and stratification of 2 months + GA3 500ppm in population 3. Germination of oriental hornbeam could be increased with stratification and GA3 treatments. Seed germination showed a decreasing trend with increase in altitude (Güney *et al.,* 2014). Furthermore, Merou *et al.* (2012) reported that *Carpinus orientalis* seeds with chemical (H_2SO_4) and mechanical scarification showed lower germination percentage. This indicates that mechanical scarification can be deadly for seeds, while chemical application can harm the embryo, further it was found the highest germination percentage was obtained by applying 40% concentration Baikal EM1 + Biohoumous mixture on seeds. Several studies have concluded that these effective microorganisms have positive effect on yield, particularly in agriculture fields (Fujita, 2000; Kremer *et al.,* 2001; Chagas *et al.,* 2011). But the natural materials have never been investigated in Turkey from the aspect of their effect on seed germination of forest tree species and development of saplings. The present study is the first one on this regard, and even 40% dose of Baikal EM1 + biohoumous mixture significantly increased the germination percentage of Oriental Hornbeam seeds.

Table 2 shows that highest germination percentage among the oriental hornbeam population was found in P17 population with 94.5%, while lowest germination percentage was seen in P6 (7.3%). On the other hand, it was found that germination percentage values varied between 40.50% and 51.55%. The level of significance was less than 0.01 from the aspect of population, pre-treatment and population (the level of confidence being 99%) and it

Table 2 : Germination percentage of population

Population	Min. germination percentage (%)	Max. germination percentage (%)	Mean germination percentage (%)
P1	8.5	83.2	45.85
P2	8.2	83.6	45.90
P3	8.3	84.2	46.25
P4	7.6	78.6	43.10
P5	7.4	78.4	42.90
P6	7.3	85.4	46.35
P7	6.8	74.2	40.50
P8	6.5	84.6	45.55
P9	8.1	84.3	46.20
P10	8.3	88.2	48.25
P11	8.8	89.5	49.15
P12	8.2	91.2	49.70
P13	8.5	92.4	50.45
P14	8.3	93.4	50.85
P15	8.4	92.5	50.46
P16	8.2	93.6	50.90
P17	8.6	94.5	51.55

Table 3 : Results of Duncan test regarding pre-treatments

Pre-treatments	Germination percentage (%)
PT1	6.88a
PT2	21.12b
PT3	16.10bc
PT4	17.81bc
PT5	25.21c
PT6	14.79b
PT7	11.76b
PT8	24.65bc
PT9	35.42cd
PT10	8.1ab
PT11	71.28de
PT12	66.25d
PT13	86.58e

revealed that all of them had impact on germination percentage.

It was found according to Duncan test that pre-treatments formed 9 different groups (Table 3). In this context, PT1 pre-treatment was placed in first group, PT13 pre-treatment was placed in final group. Baikal EM1 and biohoumous increased germination percentage in hornbeam seed.

Table 4 shows that lowest mean germination percentage was observed in P4 (Kaynaşlı), P5 (Devrek) and P7 (Kozcağız) population. Highest germination percentage was found in P17 (Hopa) population. Güney *et al.* (2014) noted highest germination percentage in Maçka 3 population and studied variations based on germination characteristics

Table 4 : Duncan test results of populations

Population	Germination percentage (%)
P1	45.85b
P2	45.90b
P3	46.25c
P4	43.10a
P5	42.90a
P6	46.35c
P7	40.50a
P8	45.55b
P9	46.20c
P10	48.25d
P11	49.15d
P12	49.70d
P13	50.45e
P14	50.85e
P15	50.46e
P16	50.90e
P17	51.55f

of *Carpinus orientalis* mill seeds that were collected from natural population at high altitudes.

Thus, in the present study sevealed that highest germination percentage was found with 40% dose of Baikal EM1 + biohoumous mixture for hornbeam seed. In this context, Baikal EM1+biohoumous effective microorganism mixture should be used for increasing germination percentage of oriental hornbeam seed in different population in Turkey.

Acknowledgments

The author is grateful to Prof. Dr. Erol Kirdar and Prof. Dr. Surhay Allahverdi for scientific suggestions. I would like to express my thanks to research persons of Forest Tree Species and Seed Improving Research Institute Directory of Turkey for seed collection of Oriental hornbeam from different populations in Turkey.

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