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Effects of cold stratification and chemical treatments on seed germination in four hazelnut cultivars

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Key words: chemical treatment, cold stratification, *Corylus avellana* L., filbert, germination.

Abstract: Propagation of European hazelnut by seed is influenced by some seed treatments. In this investigation, effect of stratification period and some chemicals on seeds of four hazelnut cultivars were studied. GA_3 and four months of stratification, each individually resulted in the highest germination percentage at 82.73% and 83.75%, respectively. There were significant differences between cultivars and treatments in terms of germination percentage and rate. The highest germination percentage and rate were observed in the local cultivar Gerd under GA_3 treatment at 100 mg/L and also after four months of stratification.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Propagation by seeds is a conventional method to produce new plants. This is one of the most recognized efficient methods which is widely applied for different plant species. Although sexual reproduction do not result in true to type plants, in breeding programs it is inevitable to apply it to grow hybrid seedlings. For example, in hazelnut, interspecific hybridization is necessary to transfer superior characters from wild species to the commercial European hazelnut (*Corylus avellana* L.) (Erdogan and Mehlenbacher, 2000).

Seed germination is a main step in plant life cycle, and is influenced by various biotic and abiotic factors (Yuan and Wysocka-Diller, 2006). It is essential to investigate different aspects in sexual propagation for all plant species. However, there are some common difficulties in using such approach to propagate many plants such as hazelnut, including seed dormancy and inconsistent seed germination which make some problems and retard improvement programs and sometimes end in hybrids loss. Therefore, studying beneficial treatments to remove dormancy and subsequent uniform seed germination is considered of great importance

(Wang and Berjak, 2000; Copeland and McDonald, 2001). Generally, germination process is controlled through a balance between inducing and inhibiting factors. Provided that concentration of inducers is higher than inhibitors, seed dormancy will predominate. Some stimulants such as temperature and light are necessary to lower the effect of inhibitors in seed. In such case, an inducing factor such as gibberellic acid (GA_3) could have cumulative influence so that germination process will commence (Bradbeer, 1988).

In many temperate zone species, dormancy prevents the seeds from germinating (Derkx, 2000). A dormant seed would not germinate even in favorable environmental conditions. Several approaches have been suggested in literature to overcome this phenomenon, including cold stratification (Bewley and Black, 1994), and seed treatment by some chemicals such as gibberellic acid (GA_3), polyamines and thiourea (Frankland, 1961; Çetinbaş and Koyuncu, 2006; Mello *et al.*, 2009). Cold stratification plays a major role as a stimulant to break seed dormancy. Also its effect is accelerated in combination with chemicals or physical removing of seed coat (Bewley and Black, 1994). This technique is usually performed at temperatures between 0 and 10°C; which vary depending on different species. However, the best reported temperature for this kind of seed treatment is 5°C (Bewley and Black, 1994). Aygun *et al.* (2009) suggested that hazelnut seeds need two to six months of pre-germination cold stratification. Dormancy in hazelnut seeds is diminished through cytological, hormonal and biochemical changes during cold stratification period. For example, mobilization of phytic acid and phosphate was observed during this treatment (Vasilios *et al*., 2005).

GA_3 treatments could remove various seed physiological dormancies and induce germination of dormant seeds (Frankland, 1961). Aygun *et al.* (2009) showed that in hazelnut seeds treated by GA_3 at concentrations from 0 to 200 mg/L, the highest seed germination percentage was obtained by 100 mg/L GA_3. The main polyamines existing in plant cells are putrescine, spermine and spermidine (Davies, 2004). Based on some evidences, polyamines have a role in seed dormancy process. Sinska and Lewandowska (1991) found that putrescine, spermine and spermidine decreased in apple seeds during cold stratification. In fact, putrescine and spermidine had inducing effect and spermine had inhibiting effect on apple seed germination. Although thiourea is not applied commonly in seed germination experiments, it is able to enhance germination of some kinds of seeds (Gul and Weber, 1998; Çetinbaş and Koyuncu, 2006). Stidham *et al.* (1980) showed that thiourea had an inducing effect on germination of 18 shrub species. According to Cetinbaş and Koyuncu (2006), this property of thiourea is attributed to its cytokinin-related effect in removing inhibitors.

This investigation aimed to study the effects of some treatments including some chemicals and cold stratification on percentage and rate of seed germination in four hazelnut cultivars grown in Iran.

2. Materials and Methods

Seeds of four hazelnut cultivars including a local cultivar Gerd and three introduced cultivars Barcelona, Ronde (= Ronde du Piemont) and Segorbe were collected from Astara Hazelnut Research Station, in Astara, Guilan province, Iran. Defected seeds were discarded and proper seeds were separated to study.

Treatments

Treatments applied in this study included GA_3 (100 and 200 mg/L), putrescine (0.01 and 0.1 mM), thiourea (1000 and 2000 mg/L) and stratification for two and four months. The control treatment was distilled water.

Germination test

The seeds were soaked for 24 hours in GA_3 (100 and 200 mg/L), putrescine (0.01 and 0.1 mM) and thiourea (1000 and 2000 mg/L), and also some seeds were soaked in distilled water. In order to prevent seeds from rotting, the treated seeds were surface sterilized with sodium hypochlorite (10% v/v for 5 min.) and then rinsed by sterilized water. Afterwards, the seeds were cultured in plastic pots containing pre-autoclaved sand as the medium (diameter of 2 mm). Germination test was conducted in a factorial completely randomized design with three treatments including “no stratification”, “two months of stratification” and “four months of stratification”. Emerging radical was considered as the index of germination. In chemical treatments, the number of germinated seeds up to 40 days from the culture date, and in stratification treatments, the number of germinated seeds up to 40 days from outing from refrigerator were recorded. Stratification treatments were just performed by keeping them in refrigerator (5°C) for two to four months. Cultured seeds were inspected regularly and in case of moisture decrease of media,

autoclaved water was sprayed on them. The measured characteristics were percentage and rate of seed germination. The following formula was used to calculate germination percentage.

Germination percentage = (number of germinated seeds / total number of seeds) × 100

Germination rate is defined as the time to reach 50 percent of germination, which was calculated by the formula below:

Germination rate = (1/time of reaching 50 percent of germination)

For each cultivar, 210 seeds were used. The data were analyzed by analysis of variance (ANOVA) and the software Germin-g was applied to measure the target parameters (Soltani *et al*., 2004).

3. Results and Discussion

According to the analysis of variance, there was a significant difference among cultivars and chemical treatments with regard to germination percentage ($P < 0.01$) (Fig. 1). Even so, no significant difference was observed among cultivars and chemical treatments regarding germination rate (Fig. 2). In addition, effect of stratification on germination rate and percentage was significantly different between the cultivars ($P<0.01$) (Fig. 3 and 4).

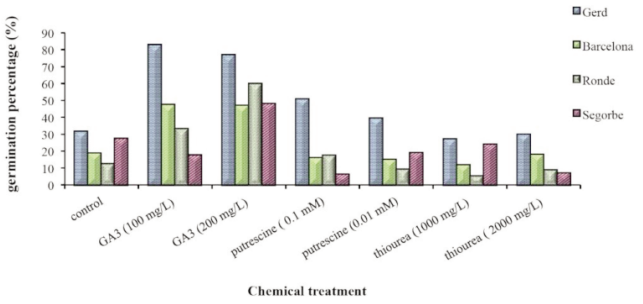

Fig. 1 - Effect of chemical treatments and cultivars on seed germination percentage of hazelnut cultivars.

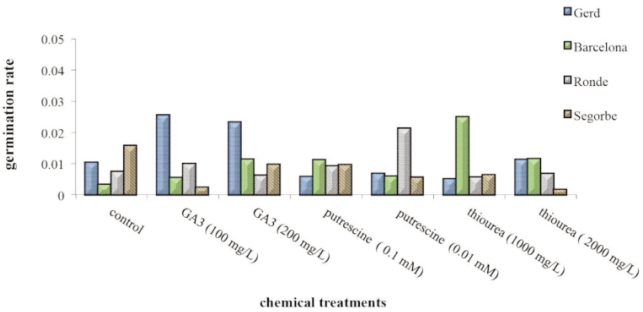

Fig. 2 - Effect of chemical treatments and cultivars on seed germination rate of hazelnut cultivars.

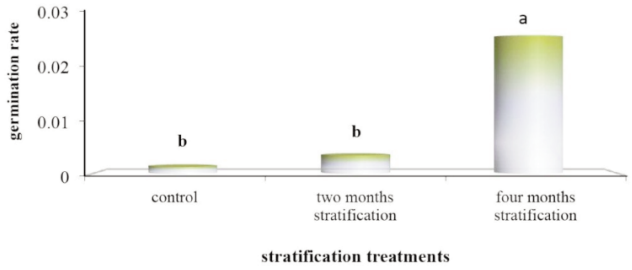

Fig. 3 - Effect of stratification treatments on seed germination rate of hazelnut cultivars.

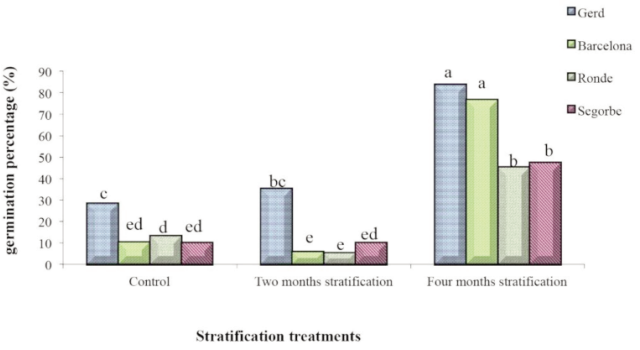

Fig. 4 - Effect of stratification treatments and cultivar on seed germination percentage of hazelnut cultivars.

For germination percentage, the difference between mutual effects of "cultivar × chemical treatment" ($P<0.05$) (Fig. 1), "cultivar × stratification" ($P<0.05$) (Fig. 4) and "chemical treatment × stratification" ($P<0.01$) (Fig. 5) was significant.

For germination rate, the mutual effect of "cultivar × chemical treatment" showed a significant difference ($P<0.05$) (Fig. 2).

Of the four hazelnut cultivars, 'Gerd' had the highest germination percentage (82.73%) at 100 mg/L GA_3, which showed 50.76% increase compared to the control (31.97%). Although applying both GA_3 concentrations was resulted in higher germination percentages, but no difference was observed between these two levels (Fig. 1).

Between stratification and cultivar, the highest germination percentage was exhibited after four

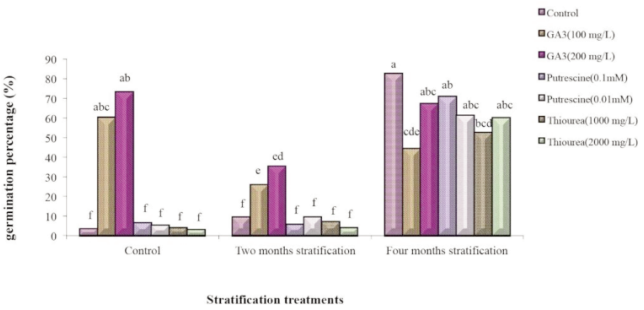

Fig. 5 - Effect of stratification and chemical treatments on seed germination percentage of hazelnut cultivars.

months of stratification in the local cultivar Gerd. No difference existed in four months of stratification between two local cultivars and Barcelona (Fig. 6).

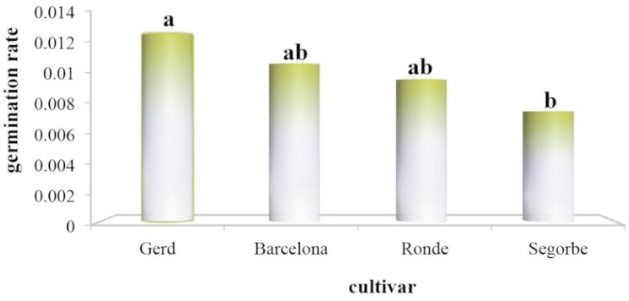

Fig. 6 - Seed germination rates in four hazelnut cultivars.

Figure 5 reveals that between mutual effect of chemicals and stratification treatments, the highest germination percentage occurred through four months of stratification and in the control treatment. Nevertheless, in treatments of two months of stratification and no stratification, both levels of GA_3 resulted in the highest germination percentage.

In all cultivars, the highest germination rate was recorded in 'Gerd' (Fig. 6). Four months of stratification had the greatest influence on germination rate (Fig. 3). Of chemical treatments, the fastest germination was obtained by using the first level of GA_3 (100 mg/L) in the cultivar Gerd (Fig. 2).

Various studies have demonstrated a direct relationship between cold stratification length and increasing in germination percentage. As can be clearly seen in figures 4 and 5, when stratification period increased, as a result, germination percentage of hazelnut seeds increased in all treatments and all cultivars. Similar results were observed by Aygun *et al*. (2009) which suggested that 120 days of cold stratification ended in higher germination percentage in hazelnut seeds in comparison with control. Furthermore, Bradbeer (1988) reported that three months of cold stratification led to a rise in hazelnut seed germination percentage. In other studies, three months of stratification without any warming improved germination percentage in seeds of *Jasminum fruticans* (Pipinis *et al.*, 2009). Another example for such impact has been reported by Chin *et al*. (1992) in kiwifruit.

In addition, there are many other studies that support the influence of applying some chemicals and plant growth regulators on rising germination percentage of seeds, which could be used singly or in combination with chilling. As a matter of fact, these chemicals are considered as substitutes for chilling requirement of seeds, and also can decrease length of chilling period.

Overall, gibberellins and cytokinins are able to promote seed germination in plants (Davies, 2004; Miransari and Smith, 2014). In contrast, abscisic acid (ABA) plays an inhibiting role in seed germination (Miransari and Smith, 2014). Among types of gibberellins, GA_3, GA_4 and GA_7 have the most impact on germination enhancement. However, cytokinins and auxins have so lower effect compared to gibberellins and cytokinins in terms of stimulating germination. In fact, the effect of all these growth regulators depends upon other factors such as light, temperature and oxygen; and also there are some mutual effects between them. Gibberellins and cytokinins are capable of neutralizing the inhibiting effect of abscisic acid. Since all these growth regulators naturally exist in seed in different ratios, so their observed effects on seed germination could be interpreted by the state of hormone balance (Kucera *et al.*, 2005).

Gibberellins are able to enhance seeds of different species to germinate through different ways. That is, external use of gibberellins could induce germination in seeds in which lack of germination is due to seed coat (e.g. legumes), or seed dormancy is because of seed embryo (e.g. apple, birch, hazelnut) (Davies, 2004; Miransari and Smith, 2014). Besides, in seeds that their germination depends on exposure to light (e.g. Arabidopsis, lettuce), GA_3 could promote seed germination even in the dark (Cao *et al.*, 2005). Based on the results obtained in this study, the highest germination percentage and rate were gained through GA_3 treatment at 100 mg/L (Fig. 1, 2), which corresponded with results reported by Aygun *et al.* (2009) that showed higher germination percentage after treatment by 100 mg/L gibberellin. In addition, there are some reports on gibberellin application on hazelnut seeds in order to enhance germination results (Bradbeer and Pinfield, 1967; Jarvis and Wilson, 1977; Pinfield and Stobart, 2006).

In addition to gibberellins, other substances such as thiourea can also overcome seed dormancy. Several investigations have demonstrated the effect of thiourea on dormancy breaking and increasing germination percentage of seeds in different plant species (Gul and Weber, 1998; Çetinbaş and Koyuncu, 2006). Also Ojha *et al.* (2010) showed that after using four treatments including gibberellin, potassium nitrate, ascorbic acid and thiourea on seeds of *Abrus precatorius*, despite increasing germination percentage in all treatments, the highest and

lowest germination percentage were obtained by gibberellin and thiourea treatments, respectively. Polyamines are also a group of growth regulators and could improve seed germination in some species (Szczotka and Lewandowska, 1989; Sinska and Lewandowska, 1991). Furthermore, in this research, putrescine had a significant difference compared to the control, in terms of germination percentage and germination rate of hazelnut seeds (Fig. 2).

4. Conclusions

The results of this study revealed that the hormone GA_3 and four months of cold stratification resulted in the highest germination percentage in hazelnut seeds at 82.73% and 83.75%, respectively. The cultivars and chemical treatments had significant effect on seed germination which were the highest in the local cultivar Gerd under GA_3 treatment at 100 mg/L and also after four months of cold stratification.

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