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Effect of Presowing Treatment with Ultrasound and Stratification of *Laurocerasus officinalis* L. Seeds on Some Growth Behaviour of Seedlings

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Abstract

This study was conducted to investigate the effects of different treatment with ultrasound and stratification of *Laurocerasus officinalis* L. seeds, conducted in greenhouse conditions during the period 2013-2015. The *Laurocerasus officinalis* L. is a species with an economic importance for the pharmaceutical industry, and it is also often used in ornamental horticulture. The determination of appropriate conditions for propagation by seeds has an effect on the cost of production. The seeds were treated with 0, 5, 10 and 5 min ultrasound and 2 months stratification. The results showed that the percentage of germinated seeds and quality root system are dependent on the exposition time. The largest number of germinated seeds was obtained with ultrasound treatment of 10 min. The stratification resulted in the obtaining of roots with the greatest length. High doses of ultrasound will provide the breaking of seeds and weak, brittle root system, which reduces the chances of successful adaptation in replanting.

Key words: Laurocerasus officinalis L., seeds, ultrasound, stratification

Introduction

Laurocerasus officinalis L. is a species in the genus *Prunus* and is widely distributed in the Mediteranean region. The plant is very often used in landscape practice in Bulgaria. *Laurocerasus officinalis* L. is evergreen, in flower from April to May and the fruits ripen in August and September. The plant tolerates light, medium and heavy soils; and can grow in heavy clay soil too (Pieroni, 2000). It can tolerate strong wings and atmospheric pollution. It can be used as tall hedge and screen mass or group in large areas and has become a popular garden plant. The flowers are very fragrant; smell sweet and delightful. It shows better pest resistance than most other evergreen shrubs (Komarov, 1968; Frohne & Pfander, 1984). This species has an important economical value for medicinal industry, too (Solusoglu et al. 2009). The fresh leaves are antispasmodic, narcotic and sedative. It can be used as a treatment for cough, asthma, dyspepsia and indigestion. A cold infusion of leaves is used as a wash for eye infection. It is a good diet fruit and has antioxidant effect (Baytop, 1963; Grieve, 1987; Chief, 1984; Pieroni, 2000; Kolayli et al. 2003).

This plant has become more popular in recent years. To use it more often we need to propagate it economically. *Laurocerasus officinalis* L. reproduces through seeds, but a lot of genetic variation occurs (Simancik, 1970; Kamenicka and Rypak, 1981). For that reason cutting propagation becomes more important. Clonally propagation of many tree and shrub species is effective through cuttings. The plant grows well on its own roots in Black Sea Region. Therefore, it can potentially be produced by rooting of cuttings. Factors such as cultivar, the collection date, degree of hardening of cuttings and the treatment concentration of auxin can affect rooting (de Olivera et al., 2003; Tsipouridis et al., 2003, 2006). Cuttings are usually made during summer months. Observation of the rooting of stem cuttings of many different genera of plants indicate that better regeneration of roots occurs when cuttings are taken either before or after, but not during flowering. Rooting followed a season trend; highest rooting occurred in other plants die to seasonal variation which brought about regeneration capacity. The regeneration capacity is low during the month of flowering (Dore, 1953; Hudson, 1953). Mackawiak (1989) obtained some roots with semi – hardwood cuttings of some *Prunus* rootstocks but failed to root with softwood and hardwood cuttings. Larsen (2008) reported that *Laurocerasus officinalis* L. could be propagated with semi – hardwood or hardwood cuttings in summer or winter seasons and have a high rooting capacity. Solusoglu and Cavusogly (2009) obtained good results with 2 g/l IBA with the springtime cuttings of *Laurocerasus officinalis* L.

According to Hartmann et al. (2002), IBA is the best auxin for general use because it is non-toxic to plants and is effective in promoting rooting of a large number of plant species.

The genetic variation probably will affect the rooting ability and species will show different characterization for rooting capacity, too. Application of synthetic auxins to shoot cuttings may be very effective in promoting root formation in some genotypes (Howard, 1967). Tsipouridis et al. (2003) found that IBA concentrations of 2000 mg/l stimulated rooting of hardwood and semi-hardwood cuttings, but rooting success varied with cultivar.

The traditional way of obtaining seedlings of fruit tree species is based on stratification of seed and black type`s seed of *Laurocerasus officinalis* L. have been known traditionally to propagate by seeds easily. Research on seed germination of *Laurocerasus officinalis* L. has not been studied before and information on germination of seeds was limited (Young and Young, 1992, Deno, 1993).

Various methods have been used by seed scientists and technologist to break seed dormancy. Gibberellic acid has been shown to break dormancy and increase germination of seeds in seeds of several genera (Onursal & Gözlekci. 2007, Demirsoy et al. 2010). Soaking in tap water or in hot water also stimulate the germination of seeds that allow gas exchange and water taking inside to seed and smoothing the seed coat too (Gerçekçioğlu & Çekiç, 1999, Onursal & Gözlekçi, (2007).

Sonification either by acoustic sound wave or by ultrasound is also a promising field in plant research and in agro and horticultural production. Sound is one of the physical signals that affect plant life although the mechanisms by which plats perceive and act upon a sound stimulus are not well known yet. There is evidence that sonication using low frequencies of sound (as little as a few dozen Hz) to as high as ultrasound (several dozen kHz) may increase organogenesis and influence growth and development of plants, but the number of studies is still limited (Teixeira da Silva et al., 2014).

It is important to understand the effect of stratification, hormonal treatment (GA_3 application), and soaking in tap or hot water on germination both for practical nursery application and for breeding studies, but the methods to stimulate germination for *Laurocerasus officinalis* L. have not been fully studied yet.

Thus the objectives of the current study were to determine germination of *Laurocerasus officinalis* L. to cold stratification treatments following a pre-treatment with sonification and to provide some practical suggestions.

Material and Methods

Mature fruits of *Prunus laurocerasus* L. were collected in August 2013 and 2014 from shrubs growing in Dendrological park in Hysaria city. The seeds were removed by hand and stored in container in refrigerator until used in the experiments. Germination experiments were started in the following September. The seed viability test made on three representative samples each including 20 seeds by the 2,3,5–tri–phenyl–tetrazolium method ISTA (1996). In the current study *Laurocerasus officinalis* L. seeds exhibit 95% viability. Firstly, seeds were surface sterilized in a 1% aqueous NaOCl (sodium hypochlorite) – solution for 5 minutes and then rinsed with distilled water three times (Cetindas & Koyuncu, 2006) and the following pre-treatment applications were used to determine the effects on germination percentage (GP) and germination rate (GR) of *Laurocerasus officinalis* L. seeds – 0, 10 and 15 min ultrasound with Ultrasonic bath (01CT405 35W, 42kHz, 220 V) and two, three and four months (60, 90 or 120 days) stratification.

All seeds were placed in moistened and stratified in small plastic boxes at 4°C in a cold storage room in Fruit Growing Institute in Plovdiv, Bulgaria.

At the end of the stratification period, seeds were sown in small boxes. The boxes were placed in greenhouse with 25°C. Germination was checked every week. The experiment lasted 6 months.

Statistical data processing was done with ANOVA test. The significant difference between the control and variants was presented as: *($P \leq 0.05$), **($P \leq 0.01$),***($P \leq 0.001$) and the non – significant as ns.

Results and Discussion

The germination percentage was significantly affected by ultrasonication. All variants increased the percentage of seed germination in comparison to control. The lowest germination percentage (37.81; 41.54; 25.73) was observed in non-treated seeds (Fig. 1.).

These results suggest that seeds of *Laurocerasus officinalis* L. have exogenous and endogenous dormancy (Simancik, 1970; Kamenicka & Rypak 1981; Demirsoy et al. 2010). The results of the study on embrio culture development of *Laurocerasus officinalis* L. supported need of dormancy. The highest germination percentage was obtained from a variant with 10 min ultrasound treatment. Increasing the period of ultrasound treatment to 15 min affected the percentage of germination negatively.

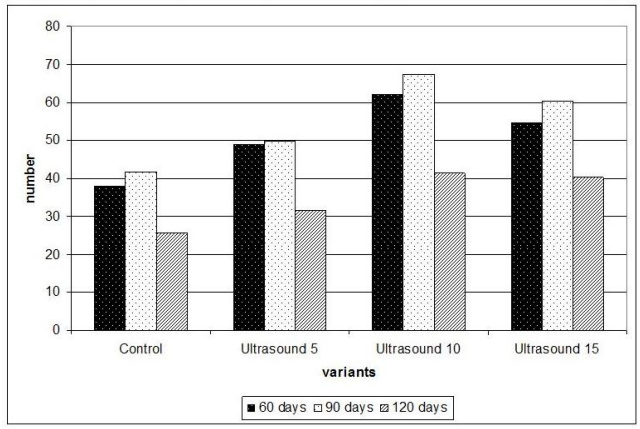

Fig. 1. The effects of ultrasonication and stratification on seed germination
Утицај ултрасоникације и стратификације на клијање сјемена

The dormancy breaking treatments is especially important to shorten the germination time. If the germination time delays, the seeds are more likely to suffer from fungal attack. On the examined indicators of the root system number of roots and root length shows that the largest number and the longest root plants treated with ultrasound for 10 min and stratified 90 days (Fig. 2.). Increasing the time of treatment with ultrasound, as well as extending the period of stratification had a negative effect.

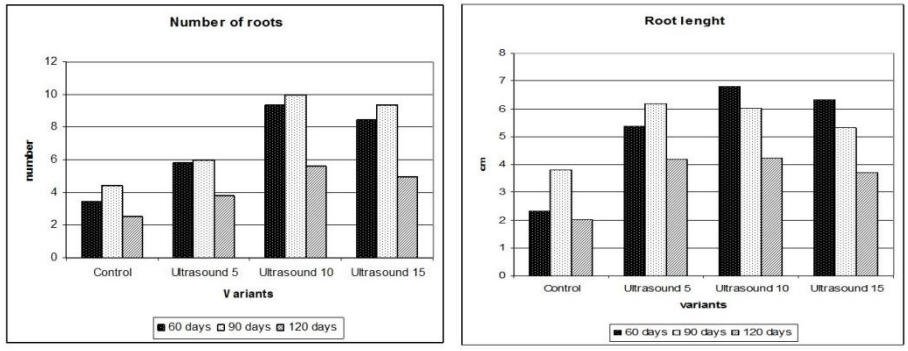

Fig. 2. The effects of ultrasonication and stratification
period on behaviour of root system
Утицај ултрасоникације и стратификације на корјенов систем

A similar trend although less pronounced is observed in the indices of stem - stem length and stem diameter, as well as those of the leaves - number of leaves and leaf area (Fig. 3. and Fig. 4.).

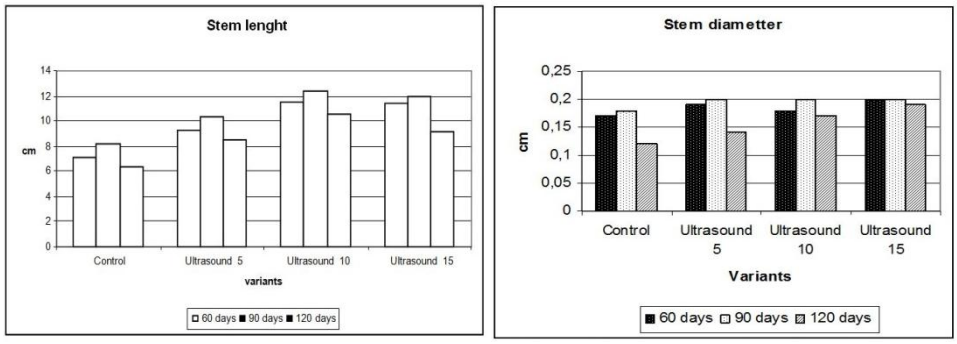

Fig. 3. The effects of ultrasonication and stratification period on behaviour of stem
Утицај трајања ултрасоникације и стратификације на стабљику

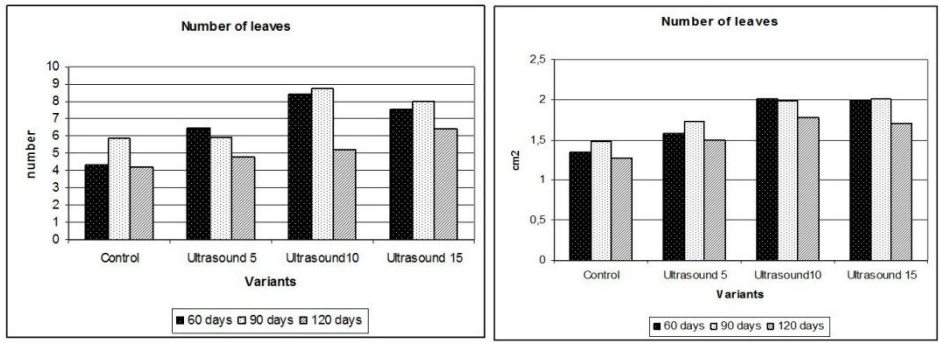

Fig. 4. The effects of ultrasonication and stratification period on behaviour of leaves
Утицај трајања ултрасоникације и стратификације на листове

Our results confirmed that a 90-day stratification and a 10-minute treatment with ultrasound was suitable for *Laurocerasus officinalis* L. and could be successfully used in commercial nursery propagation.

Conclusion

The results of the present study on the effect of pre-sowing treatment with ultrasound and stratification of *Laurocerasus officinalis* L. seeds on some growth behavior of seedlings lead to the following conclusions:

An effective way for breaking the seed dormancy and enhancing seed germination in *Laurocerasus officinalis* L. is established.

Treatment with a 10-minute ultrasound and stratification period of 90 days increased the germination rate and quality of seedlings.

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Утицај третмана прије сјетве ултразвуком и раслојавањем сјемена *Laurocerasus officinalis* L. на неке карактеристике раста сијанца

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Сажетак

Ово испитивање је спроведено да се испита утицај различитих третмана ултразвуком као и раслојавања сјемена *Laurocerasus officinalis* L., које је спроведено у условима стакленика у периоду 2013-2015. *Laurocerasus officinalis* L. је врста која има економски значај за фармацеутску индустрију, а такође се често користи у орнаменталној хортикултури. Одређивање одговарајућих услова за размножавање сјеменом има утицај на трошкове производње. Сјемена су третирана са 0, 5, 10 и 15 минута ултразвуком и у току 2 мјесеца раслојавањем. Резултати су показали да проценат проклијаних сјемена и квалитет коријеновог система зависе од времена експозиције. Највећи број проклијаних сјемена је добијен третирањем ултразвуком у трајању од 10 минута. Високе дозе ултразвука доводе до производње сјемена са слабим, крхким коријеновим системом, који смањује шансе за успјешну адаптацију на сађење.

Кључне ријечи: *Laurocerasus officinalis* L., сјемена, ултразвук, раслојавање

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