THE INFLUENCE OF DIFFERENT PRESOWING TREATMENTS ON SEEDLINGS BEHAVIOURS OF *ILEX AQUIFOLIUM* L.

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ABSTRACT

Plants from the group of evergreen deciduous shrubs used in landscaping practice in our country are not many. *Ilex aquifolium* L. is one of those plants. With its high decorative qualities and unpretentiousness to the environmental conditions, the plant deserves wider use. Problem in the production of seedlings is the deep peace of seeds and consequent low germination. This study was conducted to explore some possibilities for pre-sowing treatments of seeds of *Ilex aquifolium* L. and for increasing of their germination. They were used variations of soaking in tap, hot water and a solution of GA_3 at concentrations of 0.5 or 1,0 gl^{-1}. The seeds were stratified after different treatments. Were used seeds with or without pericarp. Stratification was done in pure washed river sand at a temperature of 4^0 C for 90 days. All treated seeds showed a higher rate of germination in comparison with the control. With the highest germination rate - 86.7 - were seeds treated with hot water and then stratified for 90 days. Treatment with GA_3 without endocarp seeds showed a higher germination rate - 74.5% and the necessity of shortening the period of stratification in comparison with seeds with endocarp. It was established that soaking in a hot or tap water and the subsequent 90-days stratification are applicable treatments that would result in practice of ornamental nurseries in our country.

Keywords: *Ilex, pre-sowing treatment, soaking, gibberellic acid*

INTRODUCTION

Ilex aquifolium L. is a species widespread in the Mediterranean region. There are many species that differ in habitus, shape and color of the leaves, flowering time and timing of seed maturation. The plant is suitable for use as ornamental in our parks and gardens, but it is not yet as widespread as it deserves. One of the reasons is the difficulties in the propagation and production of planting material. The traditional way of propagating this species is by sowing pre-stratified seeds. Studies on the germination of seeds of *Ilex aquifolium* L. are not many, and the information is limited to general conclusions and recommendations (Alcántara et al., 2000; Norman, 1993; Young and Young, 1992). Many different methods have been used by specialists to overcome seed dormancy. Gibberellic acid has been used for this purpose and to increase seed germination in some species (Andersson et al., 1998; Hartmann et al., 1975; Riley, 1987). Soaking in water, lukewarm or hot, also stimulates seed germination, allows gas exchange and water penetration through the seed coat, as well as softening of the seed coat itself and facilitating germination (Irvani et al., 2012; Nikolaeva et al., 1985; Murdoch et al.,1992). It is especially important to establish the influence of stratification, treatment with growth regulators and soaking in lukewarm (30^0C) or hot water (70^0C) on seed germination in order to use this knowledge in the practical work of ornamental nurseries for production of planting

material or in the selection to obtain new cultivars and varieties. Therefore, the aim of the present study was to establish the influence of the above factors on the germination of seeds of *Ilex aquifolium* L. and to make some practical recommendations.

Material and Methods.

Ripe fruits of *Ilex aquifolium* L. were collected in October 2017 and 2018 from plants on the territory of the Dendrological Park of the Agricultural University - Plovdiv. The fruits were picked by hand and stored in the refrigerator until use. Germination research began in October of that year. The viability test was performed on three representative samples, each containing 20 seeds of the 2, 3, 5, - tri-phenyl-tetrazolium method of ISIA (1996). In this study, the seeds of Ilex aquifolium L. showed 75% viability. The seeds were then surface sterilized with 1% Ca $(ClO)_2$ solution for 5 minutes and then rinsed three times with distilled water. The following pre-sowing treatment options were tested:

1. Control - seeds were stratified for 60, 90, 120 and 150 days and sown without pre-sowing treatment.
2. Variant 1 - soaking the seeds with endocarp in lukewarm water (30 ° C) for 5 days and stratification for 60, 90, 120 and 150 days

3. Variant 2 - soaking the seeds with endocarp in hot water (70 ° C) for 3 minutes, cooled immediately and then stratification for 60, 90, 120 and 150 days.

4. Variants - 3 and 4 - soaking seeds with and without endocarp in a solution of 0.5 gl^{-1} GA_3 for 24 hours and then stratification for 60, 90, 120 and 150 days.

5. Variants 5 and 6 - soaking the seeds with and without endocarp in a solution of 1 gl^{-1} GA_3 for 24 hours and then stratification for 60, 90, 120 and 150 days.

The seeds were placed in washed river moistened sand and stratified in plastic bags at 4 ° C in a refrigerator. At the end of the stratification period, the seeds were removed from the sand and sown in substrate in boxes. The temperature was maintained at 25 ° C and 16/8 hours photoperiod. Germination was checked every four days.

The whole experiment lasted 190 days and ended when it was found that the seeds had stopped germinating. Seeds with at least a 2 mm long root were reported as germinated (ISTA, 1996).

Results

The data in Table 1 show that the germination of *Ilex aquifolium L*. seeds is influenced by both the pre-sowing treatment and the length of the stratification period. In all experimental variants, the germination values exceeded those of the control untreated variant. In it, the germination values are in the range between 17.13% for 60-day stratification and 58.67% for 90-day stratification. With the extension of the stratification period, the percentage of germinated seeds decreases, but remains relatively high - 52.76% for 120-day stratification and 50.36% for 150-day stratification. Soaking in lukewarm water (30 ° C) for 5 days leads to a significant increase in the percentage of germinated seeds, in the variant with 60 days stratification -58.67 %, while at 90; 120 and 150 daily differences are insignificant – respectively 8.20 %, 5.14 % и 4.86 %. Soaking in hot water (70 ° C) for 3 days and 90 days of subsequent stratification leads to the highest germination of seeds of *Ilex aquifolium L*. -

93.33%. In this variant maintains the tendency to reduce the percentage of germinated seeds with increasing duration of stratification period. The differences compared to the 90-day stratification are 32.40 % for the 120-day stratification and 28.07 % for the 150-day stratification, respectively. Pre-sowing treatment of seeds with 0.5 gl^{-1} GA_3 has an extremely positive effect on germination, as the values of the different variants are in the range of 73.21% - soaking in 0.5 gl^{-1} GA_3 with pericarp at 150 days of subsequent stratification, up to 91.33% - when soaked in 0.5 $gl^{-1}GA_3$ without pericarp at 90 days stratification. Exceptions are seeds with 60-day stratification, in which the variant with pericarp germination is 21.67%, and without pericarp - 50.08%. Treatment of *Ilex aquifolium L.* seeds with 1 gl^{-1} GA_3 leads to a significant increase in germination compared to untreated seeds. The seeds with 90-day stratification have the highest values - 81.67% for seeds with pericarp and 85.07% for seeds without pericarp. Again, there is a decrease in the germination rate with increasing duration of stratification period. Germination values at 60 days stratification were 38.48% for seeds with pericarp and 41.67% for seeds without pericarp. It is noteworthy that doubling the concentration for seed treatment with GA_3 from 0.5 gl^{-1} to 1 gl^{-1} does not lead to a significant increase in the germination rate, namely the average values for the variant treated with 0.5 gl^{-1} GA_3 with pericarp is 63.42%, and in that with 1 gl^{-1} GA_3 also with pericarp - 71.82%, i.e. the difference is only 13.24 %, and the treatment with a concentration of 1 gl^{-1} GA_3 in the seeds without pericarp even demonstrates a slight negative effect - when treated with 0.5 gl^{-1} GA_3 without pericarp, the germination of the seeds is 77.06% in 69, 79% for treatment with 1 gl^{-1} GA_3 without pericarp.

Table 1. Influence of pre-sowing treatment of cold stratification on seed germination of *Ilex aquifolium* L.(%)

Variants	Duration of cold stratification (days)			
	60	90	120	150
Control	17,13	58,67	52,76	50,36
Soaking in lukewarm water (30°C) for 5 days	27,18***	63,48**	55,47*	52,81*
Soaking in hot water (70°C) for 3 days	45,18***	93,33***	70,49***	72,87***
Soaking in 0,5 gl^{-1} GA_3 with pericarp	21,67**	80,35***	78,43***	73,21***
Soaking in 0,5 gl^{-1} GA_3 without pericarp	50,08***	91,33***	86,47***	80,37***
Soaking in 1 gl^{-1} GA_3 with pericarp	38,48***	81,67***	85,57***	81,57***
Soaking in 1 gl^{-1} GA_3 without pericarp	41,67***	85,07***	78,61***	73,81***

(P≤0.05), ** (P≤0.01), *** (P≤0.001), and the unproven difference – ns

With regard to germination energy (Table 2), the data show that for untreated seeds it is quite low - from 4.28% for the stratified 150 days seeds, to 7.18% for the stratified 90 days seeds.

Table 2. Influence of pre-sowing treatment and cold stratification on germination energy of *Ilex aquifolium* L. seeds (%)

Variants	Duration of cold stratification (days)			
	60	90	120	150
Control	6,65	7,18	6,94	4,28
Soaking in lukewarm water (30 ° C) for 5 days	14,81**	9,54**	10,90*	10,74**
Soak in hot water (70 ° C) for 3 days	13,68**	19,30**	10,80**	10,21**
Soaking in 0.5 gl^{-1} GA_3 with pericarp	13,34**	12,18**	10,17*	9,89**
Soaking in 0.5 gl^{-1} GA_3 without pericarp	12,74***	9,95**	8,11**	8,27***
Soaking in 1 gl^{-1} GA_3 with pericarp	9,54***	8,07***	7,88***	7,74***
Soaking in 1 gl^{-1} GA_3 without pericarp	8,43***	7,57***	7,15***	7,98***

(P≤0.05), ** (P≤0.01), *** (P≤0.001), and the unproven difference – ns

In the experimental variants with different treatments, the germination energy is highest in seeds stratified for 60 days and varies from 8.43% when treated with 1 gl^{-1} GA_3 without pericarp, to 14.81% when soaked in lukewarm water (30 ° C) for 5 days. An exception is the variant with soaking in hot water (70 ° C) for 3 days - 19.80%, which value is actually the highest for all treatment variants and all durations of stratification period. In the case of germinating energy, there is a clear tendency to decrease its values with increasing duration of stratification period.

Discussion

The results show that the seeds of *Ilex aquifolium L*. definitely have exogenous and endogenous dormancy. The results of a number of studies by other authors on the embryoculture in *Ilex aquifolium L*. also support the claim that the seeds have a dormant period (Arrieta et al., 2004; Thompson et al.,2003; Vleeshouwers et al.,1995). Regarding the average values of germination with the highest levels is the variant treated with GA_3 in a concentration of 0.5 gl^{-1} - 77.06%, and 1.0 gl^{-1} concentration has a negative effect, but the values are significantly higher than those in the control variant. Treatment with GA_3 at concentrations of 0.5 or 1.0 gl^{-1} before cold stratification is a successful practice and results in seed disruption with or without pericarp. The results obtained are in line with the results of other researchers that combining cold stratification with GA_3 treatment leads to increased germination in a number of tree and shrub species (Baskin et al., 2001; Pickett et al., 1989; Pipinis et al., 2012). Removal of the endocarp from the seeds increases the effect of GA_3 treatment and germination increases significantly for 60 and 90 days of stratified seeds. However, for mass propagation and production of propagating material, removal of the endocarp without damaging the embryo is a rather difficult practice, and the use of GA_3 is relatively expensive. The combination of soaking in lukewarm and hot water with cold stratification increases the percentage of germinated seeds, with the highest percentage of germinated plants (93.33%) observed when treated with hot water after 90 days

of stratification. Interruption of dormancy of seeds is a very important condition for shortening the germination period (Meyer et al., 2000; Yu 2004). If germination is delayed, the seeds become more sensitive and are attacked by fungal diseases (Bewley et al., 1994; Hacker et al., 1989; Rahnama-Ghahfarokhi et al. 2007). The results show that there are significant differences in germination and depending on the duration of the cold stratification. The 60-day stratification showed the lowest germination rate in all pre-sowing treatment variants, but the values significantly exceeded those in the control variant. The germination rate increases with increasing period to 90 days and then decreases at 120 and 150 days stratification, which is probably due to damage to these seeds during the stratification period due to removal of the endocarp and soaking in water.

Conclusions

The present study creates an effective system for interrupting dormancy in the seeds of *Ilex aquifolium L.* and increase their germination. Soaking the seeds without pericarp in 0.5 gl^{-1} GA_3 for 24 hours and subsequent cold stratification for 90 days increases the percentage of germinated seeds and germinating energy. Treatment with lukewarm and hot water, followed by 90 days of cold stratification, also increases germination. These results can be successfully adapted and used in contemporary nursery practice.

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