Chapter 4

Effects of Some Hormone Applications on Germination and Morphological Characters of Endangered Plant Species *Lilium artvinense* L. Seeds

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Additional information is available at the end of the chapter

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Abstract

Lilies are economically important plants because of their large and attractive flowers. Thus, many wild species of lilies have been cultivated to produce Lilium bulbs or flowers. This work was conducted to analyse the effect of hormone applications on Lilium artvinense (Syn: Lilium ponticum K. Koch., Lilium ponticum var. artvinense (Miscz.) P. H. Davis and D. M. Hend., Lilium carniolicum var. artvinense (Miscz.) P. H. Davis and D. M. Hend and Lilium pyrenaicum var. artvinense (Miscz.) V.A. Matthews) seeds on germination percentage and seedlings morphological traits. In the research, 1000, 3000 and 5000 ppm doses of IAA, IBA, NAA and GA3 hormones were applied to L. artvinense seeds and approximately 180 days later, the number of roots, root length, offset stem height and diameter were assessed. As a result, while the control group except 5000 ppm NNA application achieved an increase in the percentage of germination (40%) of all the applications. Germination frequency up to 100% was obtained using 5000 ppm GA3. Effects of hormone applications on other key morphological characters (rooting percentage, root height, number of scions, scion height and width) are described in terms of growth rate between 1.27 and 2.44.

Keywords: *Lilium artvinense*, hormone, seed, germination, morphological characters, endangered species, root height, scion height, scion width

1. Introduction

In 2000, 47% of world population (2.9 billion people) was living in urban areas, while it is expected that 60–90% of the world's population will be living in urban areas by 2030 [1, 2]. In European countries, more than two-thirds of the whole population are living in urban areas [2, 3].

Rapid urbanization and industrialization caused human beings to distance themselves from nature more and more with each passing day and disrupted humanity's harmony with landscape. As a part of the natural world, humans have brought a piece of universe to every single place they have lived—be it a houseplant, a small garden or a carefully organized park [4]. In today's modern life, vegetation is recognized as an indicator of life quality and livability in cities [5].

In the place they grow, plants reduce air pollution [6–10], reduce noise [11], increase aesthetic value [12], have a positive psychological effect [13, 14], provide energy conservation [15], prevent erosion [16], reduce wind speed and hold the soil with their roots, thus preventing washing away of the soil with rainfalls and streams, and protect wildlife and hunting resources. Open-green areas with plantation are important activity areas for both adults and children [17, 18]. Besides, indoor plants increase the productivity of people working in the environment they grow in [19] as well as relieving physiological stress and reducing negative emotions [20–22].

Such functional use of plants led to the development of ornamental plants market, which finally has reached a point which is economically significant. In 145 countries around the world, the cultivation of ornamental plants is carried out on a total area of 220,000 ha, and the trade volume of ornamental plants is around $50 billion USD [23].

Such advancement of the ornamental plants market made the researchers to be interested in various issues such as defining the distribution areas [24, 25], protection [26–30], cultivation [31, 32], resistance to stress factors [33, 34], various applications [11, 35, 36], genetic variability [16, 37–40], their relationship with the environment [41–44], raising awareness about plants and legal dimension of the issue [45–47], thus resulting in various works on these issues. In addition to these, a large number of reports were conducted on especially the generative [48–55] and vegetative cultivation methods, and the studies are still going on [56]. However, it is of particular importance to define new species, variations, forms, cultivars or hybrids that can be launched especially to the ornamental plants market, as all plants are of high economic importance.

One of the species that can be dealt with in the ornamental plants market is *Lilium*. *Lilium* belongs to tribe *Lilieae* of *Liliaceae* and contains about 120 species [57]. Lily is one of the most important horticultural plants, and many wild species and cultivars have been cultivated for bulb and ornamental flower production [58]. Thus, many wild species of lilies have been cultivated to produce *Lilium* bulbs or flowers. Almost all cultivars of lilies are used in ornamental flower. Especially Asiatic and oriental hybrids receive a great deal of attention in the international market [59]. Due to the demand for *Lilium* in recent years, the production of *Lilium*

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has rapidly increased. For example, *Lilium* was being grown in the Netherlands in an area of 102 ha in 1960, which rose to 2412 ha in 1990 [60, 61].

However, picking especially rare and endemic species in some countries damages the populations of the species in the natural world. The easiest and the most effective way of picking the species from the natural environment is the identification of methods for producing the species in nursery conditions. If such methods are easy, cheap and practical, they can be put into practice more effectively. Seed-based production is of vital importance so as not to damage the natural populations of endangered species in particular.

'IUCN Red Data BOOK' and 'National List of European Threatened Plants List' contain three *Lilium* species from Europe. *Lilium artvinense* (Syn: *Lilium carniolicum* var. *artvinense*, *Lilium ponticum* K. Koch., *Lilium ponticum* var. *artvinense* (Miscz.) P. H. Davis and D. M. Hend., *Lilium carniolicum* var. *artvinense* (Miscz.) P. H. Davis and D. M. Hend and *Lilium pyrenaicum* var. *artvinense* (Miscz.) V. A. Matthews) [62] is one of them [63]. Exposed over a limited area, *L. artvinense* draws attention with its yellow flowers. Its natural population is severely damaged when its bulbs are picked from the natural world. To protect the natural population of the species, easy and cheap means of producing this species should be taught to locals, thereby preventing them from picking the species from the natural environment.

Previous research has mostly attempted to determine the microculture techniques of producing lilies. However, these techniques are difficult, expensive and impracticable for locals. This report examines the production of *L. artvinense* by the use of seeds.

The present review aims to define the effect of some plant growth regulators (hormones) on germination and certain morphological traits of *L. artvinense* seeds. Plant growth-regulating substances are organic chemical compounds, produced naturally in plants or applied externally, and that, even in small amounts, can have positive and negative effects on growth, development and other physiological functions, alone or in combination with other growth regulators. They can be effective in the tissue where they are synthesized, or transported to other parts of the plant and sustain this effectiveness in other organs of the plant [64, 65].

Since 1930s, plant growth regulators have been investigated for their function in agricultural products [64]. Today, there are many works with the aim to define the effects of external application of hormones commonly used to affect the growth rate and development of a plant from the stage of germination to the harvest and post-harvest treatment, and to determine proper type, concentration and time for the application of hormones. The use of hormones is so common today that they are investigated and used in agriculture, forestry and production of various plant species from ornamental to medicinal and aromatic ones [66–68].

The most relevant role of hormones is related to the control and coordination of cell division, growth and differentiation [69]. Plant hormones including ethylene, gibberellins, auxin (indole-3-acetic acid (IAA)), abscisic acid (ABA), brassinosteroids and cytokinins are biochemical substances controlling many biochemical processes and physiological in the plant [69–71]. These key molecules are produced not only by plants but also by soil microbes.

The hormones can be said to be mainly used to ensure propagation by cutting, to increase germinating power of seed, to promote and inhibit blooming, to increase plant resistance against cold, to increase seed formation in fruits, to increase fruit size, to extend fruit storage time, to increase plant resistance against diseases and pests, to control weeds, to prevent lodging in cotton and cereals, to prevent pre-harvest fruit drop, to synchronize maturity of all plants, to hasten maturity, to remove dormancy and to initiate the growth of suckers and tubers especially during the tissue culture studies [64].

Among all hormones, auxins and gibberellins are the most widely used hormones with usage rates of 20 and 17%, respectively [64]. Auxins, which were firstly used in 1929 [72], mostly cause the expansion and growth of cells and initiate cell elongation, tissue growth and root formation. Auxins are synthesized in all higher plant species, and the most commonly found auxin is indole-3-acetic acid [73].

Other commonly found auxins other than IAA are indole butyric acid (IBA), naphthalene acetic acid (NAA), naphthoxyacetic acid (NOAA), phenoxyacetic acid (POAA), 2,4-D, phenylacetic acid (PAA), para-chlorophenoxyacetic acid (4-CPA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) [64].

The oldest and most common use of growth-regulating hormones is to stimulate rooting process. Among the hormones in the auxin class, IAA, IBA and NAA are used for stimulating the rooting of cuttings. However, the most commonly applied hormone in agricultural practices is IBA [74]. IBA is applied as a highly concentrated (1000–8000 ppm) and dilute (10–250 ppm) solution. In achieving successful rooting, temperature, light conditions and water supply are also effective parameters [75].

The findings of this study are in conformity with other reports in the literature. Guney et al. [61] reported that hormone treatment caused a significant increase in the rooting percentage of *Lilium martagon* seeds, and the rooting percentage of 28.40% in the control groups could be increased with IAA, IBA, NAA and GA3 treatments. They found the highest rooting percentage in the seeds treated with IAA at 5000 ppm (86.6%).

Turhan [65] reported that the effect of hormone treatment on the rooting percentage of *L. martagon* was statistically significant. He stated that the highest rooting percentage was 66.67%, which was obtained with 3000 ppm IBA + 1000 ppm IAA treatment. In his report, the rooting percentage was found to be 61.73% with 3000 ppm IBA + 3000 ppm NAA treatment and 59.26% with 1000 ppm IBA + 1000 ppm GA3 treatment, while it was 45.83% in the control group. Sevik and Cetin [76] also reported that 3000 ppm IAA treatment in *L. artvinense* seeds increased the rooting percentage from 28.57 to 80.22%.

Rooting percentage can be said to be one of the most important morphological traits that have been examined in the researches. The main focus of the works conducted so far has been the treatments that can increase rooting percentage. The effects of auxins on rooting and plant growth have been so far widely demonstrated [77]. These hormones have been proven to increase rooting percentage in the seeds of *Robinia pseudoacacia* [78], *Pseudotsuga menziesii* [79], *Oryza sativa* [80], *Pisum sativum* [81] and many other plant species.

IBA is one of the most commonly used and studied hormones. Its impact on rooting is continuous and extremely high [82]. There are many works showing that IBA treatments alone are highly effective in increasing rooting percentage. Polat et al. [83] reported that the rooting percentage of 5% in the control group of plum cuttings increased up to 60% with 500 ppm IBA treatment and to 62.50% with 2000 ppm IBA treatment. In another work, it was revealed that the rooting percentage of 6.7% in the control group of plum cuttings increased up to 46.7% with IBA treatment [84].

Edizer and Demirel [85] reported that IBA treatments at 2000, 3000 and 4000 ppm significantly increased rooting percentage in cuttings of cherry, peach and two different plum species. They indicated that the rooting percentage of 15% in the control group of peach cuttings increased up to 80, 85 and 86.67% with IBA treatments at 2000, 3000 and 4000 ppm, respectively. In the same work, the rooting percentages of 46.67 and 60% in the control groups of plum cuttings and the rooting percentage of 48.34% in the control group of cherry cuttings were found to be 90% with 3000 ppm IBA treatment [85]. Similarly, there are studies reporting that IBA significantly increased rooting percentage in sage cuttings, and the rooting percentage of 16.25% in the control groups was increased up to 78.75% with 100 ppm IBA treatment [86].

Naphthalene acetic acid, which has a significant effect on rooting, was used in this report. It is a synthetic hormone that has been used in orcharding for many years for thinning heavy fruit set. With the effect of thinning, fruit size and quality can be increased [64]. However, the positive effect of NAA on rooting was also proven by many reports. In a study, the rooting percentage of 51.14% in the control group of *Schefflera arboricola* L. was found to have increased to 75% with 5000 ppm NAA treatment [87]. Similarly, a 6-h NAA treatment at 250 ppm was reported to increase the rooting percentage from 0 (control group) to 23% in cuttings of *Capparis sipinosa* L. [88].

Among the natural plant-growing regulators, gibberellins are the third most widely used class (estimated rate of 17%) [65], especially for horticultural applications. GA3 is mostly produced by fermentation from the fungus *Gibberella*. Today, there are approximately 100 known GA molecules, more than 50 found in plant seeds. GA3 is the most widely used one for commercial purposes [64].

There are many reports examining the effect of GA3 on rooting such as the one by Hepaksoy [89] on *Prunus avium* L. and *Prunus mahaleb* L., the work by Aygün and Dumanoğlu [90] on *Cydonia oblonga* Miller., the research by Coşge et al. [91] on *Capparis ovata* Desf. and *Capparis ovata* L., the work by Selby et al. [92] on *Picea sitchensis* (Bong.) Carr, the study by Sevik et al. [87] on *S. arboricola* L. and the research by Sevik and Guney [93] on *Melissa officinalis* L. Although it has not been determined that GA3 has a significant effect in many species, there are studies showing that it increases rooting percentage to a considerable extent [61, 94].

This work found that hormone treatments affected root number to a considerable extent. The root number, which was 1.3 in the control group, increased to 1.5 and 1.7 with 5000 ppm IAA treatment and 5000 ppm NAA treatment, respectively.

The findings of this report are in conformity with other works in the literature. Sevik and Turhan [95] reported that all hormone treatments increased the number of roots, and they found that the average root number of 0.97 in the control group of *L. martagon* was increased to 1.33 with 3000 ppm IBA + 1000 ppm NAA treatment. They also reported that the average root number of 1.14 in the control group of *L. artvinense* was increased to 2.50 with 1000 ppm IBA treatment [76]. Guney et al. [61] also reported that the root number of 1.2 in the *L. martagon* seeds increased up to 2.0 with 3000 ppm GA3 treatment.

The reports conducted on other plant species also show that root number can be increased with hormone treatments. Sevik and Guney [93] reported that the root number of 2.67 in the control group of *M. officinalis* L. was increased to 5.5 with 5000 ppm IBA treatment. Yildiz [96] also reported that the root number of 1.67 in the control group of plum was increased to the average number of 2.3 in the seeds treated with IBA, whereas Ayanoğlu and Özkan [86] indicated that the root number of 4.22 in the control group of *Salvia officinalis* L. was increased to 22.35 with 100 ppm IBA treatment. Similarly, there are studies revealing that IBA treatment can increase the number of roots four to five times in peach, plum and cherry cuttings [85]. In the work by Demiral and Ülger [97], the root number of 4.40 in the control group of cherry cuttings was increased to 16.29 with 6 mg/l NAA treatment, while in the research by Sevik et al. [87] the average root number of 5.82 in the control group of *S. arboricola* L. was increased to 9.63 and 12.33 with 1000 ppm NAA and GA3 treatments, respectively. In the report by Polat et al. [83], the root number of 0.38 in the control group of plum cuttings was increased to 10.43 with 2000 ppm IBA treatment, while in the research by Sevik and Guney [98], the root number of 4.8 in the control group of *M. officinalis* L. could be increased to 12.5 with 1000 ppm IAA treatment.

The findings of this study also reveal that hormone treatments have affected the root length of the plants to a considerable extent, increasing the root length of 27.153 mm in the control group up to 66.419 mm with 5000 ppm IBA treatment. However, more importantly, root lengths were found to be higher than those in the control groups after all hormone treatments. Therefore, the treatments can be said to have a positive effect on the root length.

The highest root lengths were obtained with 3000 ppm IBA treatment in the work by Turhan [65] on *L. martagon*, in the report by Guney et al. [61] on *L. martagon* seeds and in the research by Topacoglu et al. [99] on *Ficus benjamina* cuttings.

The works conducted show that the root length can be increased to 2.75 times in *L. artvinense* with 3000 ppm GA3 treatment [76], 16.17 times in plum cuttings with 2000 ppm IBA treatment [83], 2.34 times in cherry cuttings with 4000 ppm IBA treatment [85], 1.63 times in *S. arboricola* with 1000 ppm IBA treatment [10] and 2.26 times in sage with 100 ppm IBA [87], as compared to the control group.

Besides, results also show that, just like the root length, the highest stem height and diameter values have been obtained with 5000 ppm IBA treatment. Stem height and diameter values are 34 and 27% higher than those of the control group, respectively. Therefore, the treatments can be said not to have such a huge effect on stem formation as to make a significant difference. Similar results were also obtained from *L. martagon* seeds. The stem height of 7.16 mm in the control group of *L. martagon* increased to 8.17 mm with 3000 ppm IBA treatment, and the stem diameter of 2.93 mm increased to 3.78 with 5000 ppm IBA treatment [61]. Likewise, Turhan [65] reported that the stem number of 0.82 in the control group of *L. martagon* increased to 0.91 with 1000 ppm IBA + 1000 ppm IAA treatment, the stem height of 9.05 mm increased to 9.19 mm with 1000 ppm IBA + 1000 ppm NAA treatment and the stem diameter of 2.40 mm increased to 2.81 mm with 3000 ppm IBA + 1000 ppm NAA treatment.

However, it was indicated that, in *L. artvinense* plants treated with hormones, the stem number of 0.43 was increased to 0.92, the stem height of 1.53 mm was increased to 6.55 mm and the stem diameter of 0.97 mm was increased to 4.3 mm [76].

Apart from these, several other studies have been carried out on *Lilium* cultivation; however, most of them focus on microculture techniques. There are also works conducted on *L. davidii* var. *unicolor* [100] and *L. longiflorum* with IBA and NAA treatment [101], on *L. davidii* var. *unicolor* [102], *L. oritential* and *L. longiflorum* with IAA, IBA and NAA treatment [103, 104], on *L. longiflorum* with IAA and IBA treatment [105] and on *L. japonicum* with BA and GA3 treatment [106]. However, it is difficult to compare results of these reports with those obtained in the present work, due to rather different experimental approach.

2. Conclusions

Collecting of endangered *Lilium* species for commercial purposes is a great harm to the natural populations. The most effective way to prevent the collection of endangered species from the wild is to establish simple, cost-effective and reproducible methods of cultivation. To date, most studies focus on *Lilium* cultivation using microculture techniques, which are costly and hard to apply for the villagers who collect the flowers from the wild. Therefore, the question is still open.

This report attempted to define simple, inexpensive and effective cultivation methods for *Lilium* species. The work found that hormones applied using simple mechanisms could increase rooting process to a great extent, as well as enhancing the morphological traits of newly generated plants.

The results of this study show that all hormones have been extremely effective especially in increasing germination percentage. The germination percentage of 40% in the control group increased up to 60.66, 75.34, 82.66 and 84% in the seeds treated with NAA, IAA, IBA and GA3, respectively. Besides, 100% germination percentage was achieved with 5000 ppm GA3 treatment.

In addition, the results also reveal that hormone treatments affect each treatment differently. For example, the highest rooting percentage was obtained with 5000 ppm GA3 treatment. On the other hand, the highest number of roots was obtained with 5000 ppm NAA treatment, while the highest root length, stem height and stem diameter values were obtained with 5000 ppm IBA treatment. In practice, it would be best to use the hormone which has the greatest effect on the treatment that is desired to be enhanced.

Similar works may be repeated especially on species that are important in terms of mass cultivation, such as ornamental plants or medicinal or aromatic plants, and important data may be acquired as a result. Thus, further studies will help innovating effort, time and cost-saving practices in plant cultivation.

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