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Effects of some hormone applications on germination and morphological characters of endangered plant species *Lilium artvinense* L. Onion scales

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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. However, some non-cultivated species are still found in nature, picked and sold, which damages the natural populations of the species. The easiest and most effective way of preventing them from being nature picked is the identification of easy and cheap methods of producing them. This study attempts to determine the ways of producing *Lilium artvinense* by the use of bulb flakes. 4 hormones (Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), Naphthaleneacetic acid (NAA), and Gibberellic acid (GA3) were used, and 12 hormone applications were made. 3 doses (1000 ppm, 3000 ppm, and 5000 ppm) were used from each hormone. In addition, a control group was used. Each application was made to the basal, middle, and apical parts of the bulb flakes. In this way, 39 applications were made in total. The applications to the bulb flakes were compared in terms of 6 characters (i.e. rooting percentage, the number of roots, root height, the number of scions, scion width and height). Hormone applications can increase the likelihood of success at least two-fold, thus the species can be produced easily and cheaply.

Keywords: Danger of extinction; Auxins; Gibberellins; Likelihood; Rooting; Sustainable plant.

INTRODUCTION

Lilium belongs to the group *Lilieae* of *Liliaceae* and contains about 120 species [1]. Lilies are economically important plants because of their large and attractive flowers [2]. Thus, many wild species of lilies have been cultivated to produce lilium bulbs or flowers. Almost all horticulturalists of lilies are used to flower cutting. Especially Asiatic and oriental hybrids receive a great deal of attention at the international market [3].

However, picking especially rare and endemic species from their natural habitats in some countries damage the populations of the species in their natural environment. The easiest and the most effective way of picking these species from their natural habitats is the identification of methods of producing the species in nursery conditions. If such methods are cheap and practical, they can be put into practice more effectively. Production with seeds is of vital importance so as not to damage the natural populations of endangered species.

"IUCN Red Data BOOK" and "National List of European Threatened Plants List" contain 3 lilium species from Europe. *Lilium artvinense* is one of them [4]. Only found in a limited area, the *Lilium artvinense* draws attention with its yellow flowers. Its natural population is severely damaged when its bulbs are picked from their natural habitats. To protect the natural population of the species, easy and cheap means of producing this species should be taught to the locals, thereby preventing them from picking the species from their natural environment.

Previous research has mostly attempted to determine the microculture techniques of producing lilies. However, these techniques are difficult, expensive, and impractical for the locals. This study examines the production of *Lilium artvinense* by the use of bulb flakes.

MATERIALS AND METHODS

The bulbs belonging to *Lilium artvinense* used in the study were picked from Artvin. The bulbs picked on the 10th and 11th of July were kept by moistening them in a germination substrate. Applications were made on the bulbs brought to the laboratory on the 13th of July.

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Polyethylene tubes of 3x3x15 cm were prepared in the laboratory by filling 2/3 of them with a Klasmann sterile germination substrate. Then the bulb flakes were cut out of the basal parts via a sterile lancet. Each of the obtained nail-like bulbs was divided into 3 parts in such a way that the apical, middle and basal parts would be equal.

These 3 different parts taken from the nail-like flakes were separately subjected to the solutions of four different hormones Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), Naphthalene acetic acid (NAA) and Gibberellic acid (GA3) in 3 different doses (1000 ppm, 3000 ppm, and 5000 ppm). In this way, 13 application groups (3x4=12+1 [control] =13) were formed in total. The applications were made by sinking the samples in the hormones for 3 to 5 seconds. The same application was made 3 times in such a way that each time included 15 samples (i.e. 15 apical samples, 15 basal samples, and 15 middle samples included). 15 parts were put in each one of the prepared tubes in a way that prevented them from touching one another, covered with a germination substrate of almost 2 cm and moistened immediately.

The prepared tubes were placed in perforated boxes that did not receive direct sunlight at a room temperature of 20 to 25°C. They were irrigated every other day for 120 days. The substrates reached the saturation point during irrigation. No water accumulation occurred in the tubes because both the tubes and the boxes in which they were placed were perforated, which made the excess water flow away. Measurements were carried out on the 10th of November (i.e. the 120th day). In the meanwhile, the substrates in the tubes were discharged onto the bench. The roots were cleaned carefully. The number of roots was determined first. Then the average root height was measured via a digital micro-compass. After that, each sample was cleaned with water; the number of scions was determined and scion heights and widths were measured. All measurements were conducted through a digital micro-compass with a precision of 0.01 mm. The obtained results were entered in the table. In this way, the effects of 13 applications on the rooting percentage (RP), root height (RH), the number of scions (CCN), scion height (CCH), and scion width (CW) were evaluated.

These data were subjected to analysis of variance via SPSS 17.0. Duncan's test was carried out for those characters and found to contain a statistical difference at the minimum 95% confidence level. In this way, homogenous groups were created.

The effects of the hormone application on the rooting percentage, the number of nascent, the scions, the number of roots, root length, scion height, scion width and root height were analyzed via SPSS. The average values, analysis of variance results, and Duncan's test results concerning the characters are given in Table 1.

The values in the table indicate that the hormone applications had effects on all characters at a 99.9% confidence level. The highest rooting percentage, the highest root, the biggest number of scions, the highest scion, and the widest scion were obtained from IAA hormone applications. The largest number of roots were obtained from IBA hormone applications. Each hormone dose application was treated as a separate application in order to determine the effects of the hormone x dose interaction on the rooting percentage and the morphological characters of nascent individuals. The obtained data were analyzed. The analysis results are given in Table 2.

As seen in table 2, no rooting occurred in the 1000 ppm GA3 application and no scion emerged in the 1000 ppm NAA application. Rooting took place in all other hormone applications and the control group. The statistical analysis results indicated significant differences at a 99.9% confidence level between the applications in terms of all characters. Table 2 demonstrates that the highest roots emerged at the 1000 ppm IBA application and the highest roots were obtained at the 3000 ppm GA3 application. The highest values were obtained for IAA applications. The highest rooting percentage (80.22%) was obtained from the 3000 ppm IAA application; the largest number of scions (0.92) were obtained from the 1000 ppm IAA application and the highest (6.55 mm) and widest (4.3 mm) scions were obtained from the 5000 ppm IAA application. The obtained values indicate that IAA hormone application can increase the rooting percentage two times more than the control group. In other morphological characters, hormone applications can yield 4 to 5 times higher values compared to the control group.

The apical, middle, and basal parts were taken from the bulb flakes through division of these nail-like flakes on the metamorphic underground stem into three equal parts in order to reveal the meristematic ability of these parts. These parts' rates of forming new individuals and the morphological characters of such individuals were determined. The average values of the obtained data, F value obtained through analysis of variance, and groups formed as a result of Duncan's test are given in Table 3.

The values in table 3 indicate that the apical, middle, and basal parts have statistically significant effects on all characters of the nascent individuals including rooting percentage at the 99.9% confidence level. Duncan's test yielded two homogenous groups in terms of the rooting percentage, scion height, scion width and three homogenous groups in terms of other characters. Individuals consisting of sections taken from the basal part of the teeth were included in the first homogenous group in terms of all characters. That shows that the basal parts obtained from the nail have quite high rooting percentages, and the individuals emerging in these parts are more developed than the individuals emerging in other parts in terms of morphological characters.

It was seen in the present study that each one of the apical, middle, or basal parts obtained through the division of the nail-like flakes on the stem into 3 equal parts has potential meristematic proliferation ability. Thus, it was found out that the potential ability of each lilium bulb to form a scion may correspond to at least 3-folds of the number of nail-like flakes in the bulb. The values in the table show that while the sections obtained from the basal part have the highest values in all characters, those obtained from the apical section have the lowest values. That indicates that the meristematic proliferation ability decreases from the basal area to the apical area.

RESULTS AND DISCUSSIONS

Many studies have been conducted on the production of lilies so far. However, these studies focus on the production of lilies through microculture techniques. In these studies, IBA and NAA [5] have been used in *Lilium davidii* var. *unicolor* [6] and *Lilium longiflorum*; IAA, IBA, and NAA [7,8] have been used in *Lilium davidii* var. *unicolor* [9], *Lilium oritential*, and *Lilium longifolorum*; IAA and IBA [2] have been used in *Lilium longiflorum*; and BA and GA3 [10] have been used in *Lilium japonicum*. However, it is not reasonable to compare the results of these studies with those of the present study because of the use of different methods in them.

Table 1. The effects of the hormone applications on the rooting percentage and morphological characters.

Hormone	RP		RN		RH		CCN		CCH		CW	
IAA	70,45	d	1,84	b	73,92	D	0,87	c	5,44	c	3,43	d
IBA	54,78	c	2,20	b	69,67	C d	0,66	bc	2,86	b	1,54	bc
GA3	17,44	a	1,00	a	53,95	Bc	0,53	b	3,26	b	2,09	c
NAA	51,82	c	1,79	b	50,51	A b	0,26	a	1,12	a	0,60	A
Control	28,57	b	1,14	a	34,71	A	0,43	ab	1,53	a	0,97	Ab
F Value	40,627 ***		9,076***		7,075***		15,800***		30,924***		35,532***	

Table 2. The effects of hormone x dose interaction on the rooting percentage and morphological characters.

HOR	DOSE	RP		RN		RH		CCN		CCH		CW	
IAA	5000	56,64	ef	2,36	e	84,16	fg	0,82	cd	6,55	f	4,3	G
IBA	5000	56,64	ef	2,18	de	85,2	fg	0,91	d	3,41	cde	2,08	cde
GA3	5000	27,47	c	1,29	bc	47,23	cd	0,57	bcd	4,06	de	2,76	ef
NAA	5000	36,36	cd	1,27	bc	78,77	efg	0,27	ab	1,94	bc	0,99	ab
IAA	3000	80,22	h	1,5	bcd	74,65	efg	0,86	cd	4,82	e	3,24	f
IBA	3000	64,1	fg	2	cde	64,69	def	0,56	bcd	2,68	bcd	1,38	bcd
GA3	3000	13,85	b	1,2	b	95,72	g	0,8	cd	4,1	de	2,4	def
NAA	3000	45,06	de	2,21	de	56,32	cde	0,5	bc	1,52	ab	0,86	ab
IAA	1000	71,6	gh	1,77	bcde	64,47	def	0,92	d	5,16	ef	2,89	ef
IBA	1000	39,1	cd	2,50	e	62,92	def	0,58	bcd	2,63	bcd	1,3	bc
GA3	1000	0	a	0	a	0	a	0	a	0	a	0	a
NAA	1000	72,19	gh	1,77	bcde	20,36	ab	0	a	0	a	0	a
Cont.	0	28,57	c	1,14	b	34,71	bc	0,43	b	1,53	ab	0,97	ab
F Value		25,564***		5,837***		9,265***		8,591***		13,512***		15,872***	

Table 3. Effect of the fraction position.

Position	RP		RN		RH		CCN		CCH		CW	
Apical	6,15	a	0,60	a	20,81	A	0,07	a	0,14	a	0,11	a
Medium	27,75	b	1,32	b	50,41	B	0,25	b	1,04	a	0,46	a
Basale	67,55	c	2,14	c	72,02	C	0,76	c	4,11	b	2,50	b
F Value	281,453***		40,790***		31,296***		45,892***		56,398***		58,096***	

Auxin group hormones including IAA, IBA and NAA are intensely used in rooting works. It is reported that the use of these hormones on many species including *Melissa officinalis* [11], *Robinia pseudoacacia* [12], *Pseudotsuga menziesii* [13], *Oryza sativa* [14] and *Pisum sativum* [15] can increase the rooting percentage. Gibberelins are the third most commonly used group among the plant growth regulators [16]. Although GA3 is mostly used for enhancing flower yield or plant growth [17], it also increases the rooting percentage [11,18].

CONCLUSIONS

Picking the endangered Lilium species from the nature and selling them damage the natural populations of these species badly. The most effective way of preventing them from being picked is the identification of methods of producing them in simple environments free of cost. The previous research has mostly aimed to determine the methods of producing lilia through microculture techniques. However, these methods are too difficult and costly to be implemented by villagers who pick them. Thus, they are ineffective for the solution of the problem.

This study made an attempt to identify the methods of producing lilies by use of bulb flakes in a simple, cheap, and effective way. It was found out that the hormones, which can be applied via very simple mechanisms, increase rooting success substantially and improve the morphological characters of the nascent individuals considerably. Especially IAA application can increase the rooting percentage minimum two-fold.

Since plants are vegetatively produced by the use of bulbs, the genetic structure is safeguard. Thus, the new plants to be produced will be genetically exactly the same as the rootstock plants. In this sense, when the individual characteristics desired have certain features (e.g. high flower yield, large flower formation, flowers in particular color tones, individuals resistant to stress conditions) are produced vegetatively, it is guaranteed that the desired features are maintained.

The present study showed that a minimum of 150-200 individuals can be produced out of a bulb of normal size. However, only the bulb flakes outside the bulk should be taken without picking bulbs completely, and their internal parts should be left as they are so that these species do not disappear, and the natural existence of these populations is not damaged. At least 2-3 individuals can be produced out of one flake by cutting the collected bulb flake with a clean and sharp object.

In the present study, the greatest deal of rooting occurred for a 3000 ppm IAA application; the highest roots were formed for a 1000 ppm IBA application; the biggest number of scions were obtained for the 1000 ppm IAA application; and the highest and the widest scion formation took place for the 5000 ppm IAA application. Based on these results, the hormone application needed for the desired character to come to the forefront may be chosen. For instance, a 3000 ppm IAA application may be used for a high rooting percentage; a 1000 ppm IBA application may be used for high root formation; and a 5000 ppm IAA application may be used for bigger scion formation.

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ЕФЕКТИ НА НЯКОИ ХОРМОНИ ВЪРХУ ЗРЕЕНЕТО И МОРФОЛОГИЧНИТЕ ХАРАКТЕРИСТИКИ НА ЗАСТРАШЕНИЯ РАСТИТЕЛЕН ВИД *Lilium artvinense* L. ЛУКОВИЧНИ ЛЮСПИ

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(Резюме)

Лилиите са икономически важни растения поради техните големи и привлекателни цветове. При това много диви видове от лилиите се култивират за производството на луковици и цветя. Но има и диво-растящи лилии, които се берат и продават, като с това се нарушават естествените им популации. Най-лесният и ефективен начин това да се предотвратии е идентификацията на лесен и евтин начин за производството им. В тази работа се прави опит да се намери начин за производството на *Lilium artvinense* използвайки луковични люспи. Използвани са четири хормона (индол-3-оцетна киселина (IAA), индол-3-маслена киселина (IBA), нафтален-оцетна киселина (NAA) и гибелеринова киселина (GA3) и са приложени 12 пъти. Използвани са по три дози (1000 ppm, 3000 ppm, и 5000 ppm) от всеки хормон. Отделно е изпитана и контролна група. Всяко прилагане се прави на основната, средната и апикалната част на луковичните люспи. По този начин са направени общо 39 наблюдения. , In addition, a control group was used. Резултатите с луковични люспи са сравнени по шест показателя (процент на корени, брой на корените, височина на корените, брой на калемите, тяхната широчина и височина). Прилагането на хормони може да повиши успешните опити поне двойно, като при това растението се произвежда лесно и евтино.