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ORIGINAL ARTICLE

Investigating the Propagation of Seedless, Seeded and Ornamental Barberry Seedlings through Tissue Culture

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KEYWORDS

Barberry;
Tissue Culture;
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ABSTRACT: In order to investigate the reproduction of seedless, seeded and ornamental barberry shrubs through tissue culture, a factorial experiment in the form of a completely randomized design in three replications and with three factors, the first factor of three types of barberry shrubs (seedless, seeded and ornamental), the second factor of BAP hormone at four levels (0, 1, 2, 3 mg l^{-1}) and the third factor of IBA hormone at two levels (0 and 0.25 mg l^{-1}) was done in the laboratory of Islamic Azad University, Damghan branch in 2019. The culture medium used was based on Murashige and Stooge (MS). After preparing the culture medium and sterilizing it by autoclave, it was distributed in the test tubes. After preparation, the samples were cultured in a sterile environment and then the samples were stimulated to grow using growth hormones. The investigated traits included germination, number of leaves, length, width and surface of leaves, ratio of width to length of leaves, fresh weight and dry weight of leaves. After collecting information, the data obtained from the experiment was organized and then analysis of variance was performed with SAS statistical software and comparison of means with Duncan's multi-range test . Pearson's correlation coefficient was used in order to determine the relationships of traits. The main effects and some interaction effects on the time of greening, number of leaves, length, width, leaf area and leaf width to length ratio, leaf fresh weight and dry weight were significant. The correlation coefficient shown between the dry weight of leaves with the number of leaves ($r=0.88^{**}$), leaf length ($r=0.74^{**}$), leaf width ($r=0.74^{**}$), leaf area ($r=0.75^{**}$), green leaf weight($r=0.96^{**}$) positive correlation was significant at 1% probability level.

INTRODUCTION

In the land of Iran, there are many talents related to the production of horticultural and agricultural products, in this regard, more attention should be paid to the products whose origin is Iran, including the barberry, which is cultivated due to the lack of rainfall. In most regions of Iran and the limited water resources and the work of low-expectation products is very important [1]. Considering the size of the country and having unsuitable soil for trees and unsuitable conditions, what kind of crimson can be done in the water and soil of many areas that are not suitable for other agricultural products. These areas can be dedicated to the cultivation of seedless barberry shrub [2].

Barberry with the scientific name Berbers vulgaris from the genus *Berberis L*. Belonging to the Berberidaceae and *Ranunculaceae* families and in the dicotyledonous and diploid category and having 28 chromosomes, it is also called polyploidy, which probably originates from diploids [3].

The barberry genus has more than 660 species, of which only one type, the seedless barberry (Berberis vulgaris var. asperma), is cultivated as a garden product in

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Khorasan [4]. According to the reports available in the provinces of East Azerbaijan, Fars, Kerman, Gilan and the northwestern provinces, parts of Kurdistan, Isfahan, Yazd and around Tehran, etc. have also been reported [1].

At present, the barberry shrub is seedless with an area of 14146 hectares of fertile gardens, 14132 hectares of irrigated and 14 hectares of dry land, and 3063 hectares of non-fertile gardens, of which 2909 hectares are irrigated and 154 hectares of dry land, and the total cultivated area is 17209 hectares in the country.

be which is known with the production of 19124 tons of dry products (equivalent to about 60000 tons of wet products) per year and the total value of the production at a price of 50 per kilo tomans is about 956200000000 and if this product gains its real position in the export, it will have an impact It will be more noticeable on the country's economy. During the past years, that is, from 2001 to 2018, the area under seedless barberry cultivation has increased from 6275 hectares to about 17209 hectares, which indicates a tripling of this level. Meanwhile, barberry production has increased from 7000 tons to about 19124 tons per year. By comparing these two numbers, it can be concluded that the increase in production in this plant was due to the increase in the area under cultivation, and the increase in yield per unit area has not played a significant role in increasing the production of this product so far. Therefore, the cultivated area, the amount of production and yield of barberry in the country by province are reported in (Table 1).

Table 1. Cultivated area, production and yield of barberry in the country by provinces

Province	Infertile surface (hectares)			fertile surface (hectares)			The whole surface	Production amount (tons per hectare)			yield (tons per hectare)	
	Irrigation	Rainfed	Total	Irrigation	Rainfed	Total		Irrigation	Rainfed	Total	Irrigation	Rainfed
East Azerbaijan	5	0	5,0	25	0	25	30	36	0	36	1,444	-
Ardabil	6,6	0	6,6	0,5	0	0,5	7,1	0	0	0	-	-
Esfahan	10	0	10	11	0	11	21	11	0	11	1,000	-
Alborz	1,1	1	2,1	6,5	0	6,5	8,6	15	0	15	2,334	-
Tehran	0	5,0	5,0	0,1	0	0,1	5,1	0,25	0	0,25	2,500	-
South Khorasan	2,703	147	2,850	13,877	0	13,877	16,727	18,830	0	18,830	1,357	-
Khorasan Razavi	29	0	29	127	0	127	155	125	0	125	984	-
North Khorasan	10	0	10	23	0	23	33	9,20	0	9,20	400	-
Semnan	23	0	23	20	0	20	43	31	0	31	1,599	-
Sistan and Baluchistan	8,0	0	8,0	4,5	0	4,5	13	2,44	0	2,44	542	-
Fars	3,0	0	3	1,4	0	1,4	4,4	3	0	3	2,143	-
Kurdistan	2,0	0	2,0	2,0	0	2,0	4,0	5	0	5	2,500	-
Kerman	94	0	94	18	0	18	112	26	0	26	1,500	-
Kermanshah	0,1	0	0,1	0	0	0	0,1	0	0	0	-	-
Golestan	0	0.8	0.8	0	0	0	0.8	0	0	0	0	-
Guilan	0	0	0	1,5	14	16	16	5	16	18	1,330	1,150
Lorestan	8,0	0	8,0	12	0	12	20	9	0	9	750	-
Markazi	0	0	0	1,0	0	1,0	1,0	1	0	1	1,000	-
Yazd	7	0	7	2.5	0	2.5	9.5	1.63	0	1.63	651	-

http://www.agriis.ir/doc/45543/ Statistics letter of the Ministry Jihad and agriculture [25].

Varieties of barberry

Varieties of barberry in Iran include Hawthorn barberry (*B. crataegina*), Zarafshani barberry (*B. integerrima*),

Khorasani barberry (*B. khorasanica*), cluster barberry (*B. orthobotrys*), common barberry (*B. vulgaris*), and Japanese barberry (*B. thunbergii var.atropurpurea*) [5].

Considering that the vast plateau of Iran is considered one of the natural centers for the growth of fruit trees, despite the history of growing fruit trees in Iran, today we have benefited less from the advances in horticultural methods in the world. As a result, our country, having diverse and suitable climatic conditions, has not been able to gain a worthy position economically among fruit-producing countries [6]. Despite the history of growing seedless barberry in our country for several centuries, we still have not benefited much from new science in the production of this shrub. The remarkable jump that happened in the performance of most agricultural and horticultural crops in the second half of the 20th century has not been seen in the case of seedless barberry shrub. Such a situation reduces the economic competitiveness of seedless barberry with crops and other garden products of other regions, therefore, a scientific approach to the production of seedless barberry and research on this crop is inevitable [1]. Today, seedless barberry is propagated by rooting, and this propagation method has some limitations. But other types of barberry (seeded and ornamental) are propagated by seeds, planting, grafting and cuttings. Vegetative propagation, which includes cuttings, planting and grafting, has been important in agriculture for a long time and it is used in the propagation of crops, fruit trees, etc. Of course, vegetative reproduction methods in natural conditions are not effective in some cases and a new method of plant cultivation has been introduced and presented under the title of plant cell and tissue culture. It is also mentioned and it is used for all types of sterile cultures that are carried out in vitro. Currently, tissue culture techniques have become a powerful tool for studying basic and applied problems of plant biology.

Since the distant past, barberry has often been propagated by cuttings and cuttings, which took a lot of time, but by using the tissue culture method, rapid propagation can be done in an in vitro environment [4]. Nowadays, research has been done on the use of tissue culture in the field of reproductive roots and reproductive branches and cell purification for the production of different types of barberry. The results of experiments on the rooting of Indian barberry showed that the highest number of roots in the cutting and the highest root length and rooting percentage of 86.66% in 1992 and 83.33% in 1993 were obtained from the treatment of 500 parts per million IBA [7]. In a research conducted on Japanese barberry cuttings (B.*thunbergll*) obtained from mother plants grown in 37% to 100% full light conditions and treated with IBA hormone (1%, 3% and 8%) in the misting system. were placed alternately, it showed that the cuttings of this species and its variety "Atroporpure A" had better rooting in the treatment of 7% sunlight on mother plants [8]. The result of a research on B. trifoliata species using woody plant root culture medium indicates that the age of the branch has no effect on the number of shoots in small samples, but it has an effect on the generative root [9]. The results of another study showed that in B. thunbergll species, the use of 2 mg l^{-1} of benzyl adenine has accelerated and stimulated lateral branching, and the best result of the root formation of small branches was obtained from the culture medium without auxin after stimulation with auxin [10]. On the other hand, the use of woody plant cultivation medium (WPM) with light has a positive effect on the branching of cultivated samples, as well as small samples with two nodes compared to single nodes and those that were placed vertically on the surface of the culture medium. , had more reproductive branches [11].

Sazmand *et al.*, in a report reported that MS medium containing 1.1 mg l^{-1} BAP is the best medium for direct regeneration of barberry, and MS medium containing 2 mg l^{-1} BA + 0.01 mg l^{-1} is the best medium for barberry processing [4]. Salami (2014) investigated the effect of different plant growth regulators in the micro propagation of seedless barberry in an experiment and stated that this research was conducted in four separate experiments and the experiments were conducted in a factorial format in a completely randomized design. Sterilized lateral buds have been used as explants. The explants were transferred to culture media containing different amounts of Kin, BA, TDZ, 2ip and Z regulators. The least contamination was observed in the explants treated with 0.5% binomial (10 minutes) + 70% alcohol (1 minute) and 0.1% mercury chloride. (8 minutes) were disinfected and observed. The results indicated that the WPM medium containing 1.5 mg l^{-1} of

BA was the best medium for seedless barberry regeneration. It was also determined that the explants with two buds had better growth and regeneration than one bud. In general, it was concluded that two-node explants along with BA growth regulator are the most effective factors for seedless barberry regeneration [12].

Sazmand *et al*. investigated the regeneration using the combination of different hormones BA, GA3, IAA, BAP, Kin, IBA in MS and B5 base medium for barberry and reported that among the collected explants the ones that were collected from the beginning of May to the end of June had the highest regeneration. Also, the results showed that the best treatment for disinfecting the explants is 3% sodium hypochlorite solution for 15 minutes and then washing 3 times with sterile distilled water each time for 5 minutes. Minutes and MS medium containing 1.1 mg l^{-1} BAP is the best medium for direct regeneration of barberry. MS medium containing 2 mg l^{-1} BA 2 mg l^{-1} IBA + introduced the best barberry processing medium [13].

Ziaratnia and Salmani in a research with the aim of optimizing effective factors in callus formation of barberry from 24 combinations of growth regulators including 2, 4-D and NAA at the rate of 2 and 4 mg l^{-1} along with BA and Kin at the rate of 1, 4 and 8 mg l^{-1} in B_5 culture medium were used to induce callus and reported that NAA 2 mg l^{-1} along with Kin 1 mg l^{-1} is the best hormonal combination in callus formation in B_5 culture medium. In order to prevent browning of explants, washing with sterile distilled water for one hour after sterilization and using phenol absorbent compounds such as PVP and PVPP [14].

In a research, Mackey *et al.* succeeded in cultivating B. *trifoxila* barberry tissue using modified WPM culture and MS culture medium vitamins [15].

In a research, Arina *et al.* suggested replanting once every three days and using plant growth regulator BA up to 80% to prevent tissue browning. Also, by using the modified MS culture medium, they succeeded in growing barberry B. *Buxifolia* [16].

In a research, Karhu and Hakala used dark periods to control phenolic substances and rooting and succeeded in the micro-growth of barberry B. *Thubbergi.* They used apical buds in their research and reported that this plant tissue showed the best response to the culture environment [10].

Sazmand *et al*., investigated the effect of different plant growth hormones on the callus formation of barberry and used 1 cm long side shoots as micro-samples. After different stages of sterilization, the micro-samples were transferred to callus. Formation media containing different amounts of GA3, IAA, BAP, BA, and Kin hormones [12]

Their results showed that MS medium containing 10 micromole/liter BAP is the best media for callus formation. Barberry

In a research, Sazmand *et al.* Investigated the effect of different plant growth hormones on the callus formation of barberry and used lateral buds 1 cm long as micro-samples. After different stages of sterilization, the micro-samples were transferred to callus formation media containing different amounts of GA3, IAA, BAP, BA, and Kin hormones. Their results showed that MS medium containing 10 m mol l^{-1} BAP is the best media for barberry callus formation [13]

. In a study, Ziaratnia *et al*. showed that hormonal treatments containing Kin, NAA and BN were effective in inducing callus, so that callus formation was observed with different frequencies in most of the treatments. Also, the best hormonal combination for callus induction in barberry was 2 mg l^{-1} NAA and 1 mg l^{-1} Kin [14].

In a study, plant micro propagation Mahonia soft caress From the Berberidaceae family from the basic culture medium B_5 together with 30 g L^{-1} sucrose and 5 mg g^{-1} BAP hormone, 5 mg g^{-1} kinetin, 0.5 mg g^{-1} IAA and 2.5 mg gibberellic acid is known as the best culture medium for propagation. But 8 mg of BAP hormone showed a significant effect on rooting percentage [17]. Sazmand and Safarnejad reported that BAP hormone is better than the hormones used in regeneration in the study of callus induction and breeding of Berberis vulgaris under in vitro conditions [18].

In a research by Sazmand *et al*., the effect of growth regulating hormones on direct regeneration in barberry Berberis vulgaris var. Asperma studied in vitro and regeneration was investigated using the combination of different hormones BA, GA3, IAA, BAP, Kin, and IBA in MS and B5 basic culture medium. Young shoots and lateral shoots were used as explants. After sterilization, the explants were transferred to the regeneration

medium. The cultures were placed in the growth chamber at a temperature of 25 ± 2 °C and 16 hours of light and 8 hours of darkness. In order to remove phenol, multiple cultivations were done and after about two weeks, direct regeneration was observed. Among the explants collected, the explants that were collected from early May to late June showed the highest regeneration. Also, the results showed that MS medium containing 1.1 mg l^{-1} BAP is the best medium for direct regeneration of barberry, and MS medium containing 2 mg l^{-1} BA + 0.01 mg l^{-1} IBA was recognized as the best medium for barberry cultivation [13].

In an experiment, Mazeginejad investigated the in vitro viability of *Berberis vulgaris* L. calluses and in the first experiment, the combination of hormones suitable for the induction and growth of callus from barberry leaves and environment (MS/2+ 0.5 mg l^{-1} 2-4-D + 2 mg l^{-1} BAP) He reported the best combination of hormones. In another experiment to determine the optimal culture medium for the survival of the two hormones BAP (2 mg) and TDZ (15 mM) separately in combination with each of the factors methyl jasmonate (1 and 2 mg), iron oxide nanoparticles (75 and 100 ppm) and used zinc oxide nanoparticles (75 and 100 ppm). Finally, the external observation of callus viability showed that treatments containing BAP had higher viability than TDZ treatments in terms of appearance. So that in the treatment of BAP and Nano zinc oxide with a concentration (75 ppm) 100% survival was reported. In general, the evaluation of calluses reported the high performance of nanoparticles, especially zinc oxide nanoparticles, on the reduction of phenolic compounds and browning [19].

In a research, Mohammadi investigated the in vitro culture of seedless barberry (*Berberis vulgaris* var. asperma) and for callus formation from explants of leaves, one-year stems and buds in WPM, B_5, MS, $MS_{2.1}$ and $MS_{4.1}$ culture media. Along with the growth regulators 2, 4-D, NAA, IBA, IAA and picloram in concentrations of 2, 5 and 10 mg l^{-1} were used to induce callus. In order to investigate the effect of the season on callus formation and browning of explants, sampling was done from the middle of April to September. For direct micro propagation, the green shoots of the current year were used, and for micro grafting, one-year-old and current-year seedless barberry stems were used on four bases of the species available in the barberry collection of Pajeh School of Science and Food Industry, located in Kherson Science and Technology Park. Became. The test results showed that the best treatment to prevent explants from turning brown is washing with sterile distilled water for one hour after surface sterilization, and the best explant for callus formation is leaf explants. Complete MS culture medium with 8 g L^{-1} agar was the best culture medium for seedless barberry callus formation. The callus formation of explants harvested in the months of May and June was higher than in other months. Growth regulators 2, 4-D, NAA and picloram with a concentration of 10 mg l^{-1} had the greatest effect on callus formation. Internal contamination was not observed in bud and leaf explants from April and May, but it was observed in leaf explants from June to September and one-year stem explants. It was not possible to culture the tissue of the main branches of the current year due to contamination and necrosis of the explant, and transplanting faced serious problems due to severe contamination in the explants of the current year and one-year-old explants [20].

Since the results of the conducted research have identified the rooting problem of seedless barberry cuttings, little success has been achieved in rooting its cuttings [2, 4 and 28]. On the other hand, so far there has been no report of successful micro propagation in seedless rosacea and the only available way to increase its propagation was through rooting [1]. Therefore, taking into account that barberry is often propagated through basal shoot, which takes a lot of time, if successful; tissue culture can be used for rapid propagation in an in vitro environment. So far, there has been no report of successful tissue culture in barberry.

MATERIALS AND METHODS

This article is extracted from research project number 1429906230013.

In order to investigate the reproduction of seedless, seeded and ornamental barberry shrubs through tissue culture, a factorial experiment in the form of a completely randomized design in three replications and with three factors, the first factor of three types of

barberry shrubs (seedless, seeded and ornamental), the second factor of BAP hormone at four levels (0, 1, 2, 3 mg l^{-1}) and the third factor of IBA hormone at two levels (0 and 0.25 mg l^{-1}) was done in the laboratory of Islamic Azad University, Damghan branch in 2019. The culture medium used was based on Murashige and Stooge (MS). After preparing the culture medium and sterilizing it by autoclave, it was distributed in the test tubes (Figure1).

After preparation, the samples were cultured in a sterile environment and then the samples were stimulated to grow using growth hormones. The investigated traits included germination, number of leaves, length, width and surface of leaves, ratio of width to length of leaves, fresh weight and dry weight of leaves. After collecting information, the data obtained from the experiment was organized and then analysis of variance was performed with SAS statistical software and comparison of means with Duncan's multi-range test . Pearson's correlation coefficient was used in order to determine the relationships of traits.

So, the samples were prepared and sterilized and their cultures were then transferred to the growth chamber and placed under cold white fluorescent lamps at a temperature of 21 degrees Celsius and light conditions of 16 hours of light and 8 hours of darkness, and then the samples were treated with growth hormones. They were stimulated to grow. In this study, the characteristics of germination, number of leaves, length, width and surface of leaves, ratio of width to length of leaves, wet and dry weight of leaves on day 30 were (Figures 2 and3).

Figure 1. From right to left, grainy, ornamental and seedless barberry tree.

Figure 2. View of the incubator device.

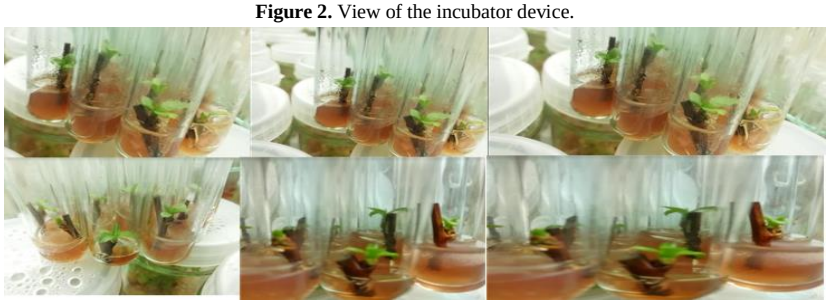

Figure 3. Growth process of samples in tissue culture medium.

Statistical analysis

After collecting the information, the data obtained from the organization test and then analysis of variance and comparison of averages were performed by SAS statistical software using Duncan's multi-range test, and in order to determine the relationship of traits, Pearson's correlation coefficient was used to draw a graph from the software. Excel software was used.

RESULTS

Analysis of variance showed that the effect of BAP hormone on germination time, number of leaves, leaf width, leaf length, leaf area, leaf width to length ratio, fresh weight of leaves, and dry weight of leaves was significant at 1% probability level (Table 2). At the concentration of 3 mg l^{-1}, the germination, number of leaves, leaf width, leaf length, leaf surface, fresh weight of leaves, and dry weight of leaves showed superiority over the rest of the BAP hormone values, but the ratio of leaf width to length showed superiority in the concentration of 1 mg l^{-1} of BAP hormone (Table 3). The effect of IBA hormone and the effect of barberry shrub type on germination time, number of leaves, width, length and surface area, leaf width to length ratio, fresh weight and dry weight of leaves were significant at the 1% probability level (Table 2). At the concentration of 0.25 mg l^{-1} the germination time, number of leaves, width, length and surface area, fresh weight and dry weight of the leaves showed superiority over the absence of IBA hormone, but the ratio of leaf width to length at the concentration of 0.25 mg l^{-1} of IBA hormone was superior (Table 3). The effect of the type of barberry shrub on the germination, number of leaves, width, length and surface of leaves, ratio of width to length of leaves, fresh weight and dry weight of leaves was significant at 1% probability level (Table 2). In terms of germination, number of leaves, width, length and surface of leaves, ratio of width to length of leaves, fresh weight and dry weight of leaves, ornamental barberry showed superiority, but in terms of number of leaves, seedless barberry showed superiority (Table 3). The interaction effect of BAP hormone × barberry shrub type on width, length and leaf area, leaf length to width ratio, fresh weight and dry weight of leaves was significant at the 1% probability level, but it had no significant effect on the germination and the number of leaves. It did not have any (Table 2). In terms of leaf width, length and surface area, the ratio of leaf width to length, fresh weight and dry weight of leaves was observed to be superior in the interaction effect of ornamental barberry shrub and concentration of 3 mg l^{-1} of BAP hormone (Table 4).

Table 2. Analysis of variance the effect of barberry shrub type and BAP and IBA hormones on the studied variables.

Sources of variation	Degrees of freedom	Mean of square							
		Dry weight of leaves	Fresh weight of leaves	Leaf width to length ratio	leaf surface	leaf length	leaf width	number of leaves	Germination
BAP	3	71.25**	0.0065**	8299.41**	120.84**	23.00**	17.93**	2.67**	2.67**
IBA	1	18.94**	118.32**	0.0016**	10097.46**	139.54**	22.61**	23.60**	10.74**
Barberry type	2	0.62**	3.95**	0.01**	1213.99**	6.44**	5.58**	3.63**	5.91**
Barberry type *BAP	6	0.15**	0.95**	0.0043**	241.22**	1.76**	1.21**	0.70 ns	0.13 ns
Barberry type *IBA	2	0.64**	3.98**	0.0048**	108.73**	1.28**	0.76**	1.92**	0.15 ns
IBA*BAP	3	0.92**	5.73**	0.0020**	407.16**	7.27**	0.38**	1.59**	1.49**
Barberry type *IBA*BAP	6	0.12**	0.73**	0.0035**	97.42**	1.81**	0.55**	0.31 ns	0.19 ns
Error	48	0.04	0.24	0.0002	18.19	0.19	0.08	0.33	0.2
Coefficient of variation (CV)		10.46	10.49	3.43	6.26	2.84	4.17	8.65	11.95

ns, * and ** are not significant, significant at 5% and 1% level, respectively.

The interaction effect of IBA hormone × type of barberry shrub on the number of leaves, width, length and surface of leaves, ratio of width to length of leaves, fresh weight and dry weight of leaves was significant at the 1% probability level, but it had no effect on the time of greening. It was not significant (Table 2). In terms of the number of leaves, width, length and surface of leaves, the ratio of width to length of leaves, fresh weight and dry weight of leaves were observed to be superior in the interaction effect of ornamental barberry shrub and in the concentration of 0.25 mg l^{-1} of IBA hormone (Table 4). The double interaction effect of IBA × BAP hormone on germination time, number of leaves, width, length and surface of leaves, ratio of width to length of leaves, fresh

weight and dry weight of leaves was significant at 1% probability level (Table 2). In terms of the number of leaves, width, length and leaf surface, the ratio of width to length of leaves, fresh weight and dry weight of leaves in the double interaction effect of IBA × BAP hormone was observed to be superior in the concentration of 0.25 mg l^{-1} of IBA and in the concentration of 3 mg l^{-1} g of BAP. Table 4). The triple interaction effect of IBA hormone × IBA × type of barberry shrub on width, length and leaf area, leaf width to length ratio, fresh weight and dry weight of leaves was significant at the 1% probability level, but on the time of greening and the number of leaves There was no significant effect (Table 2).

Table 3. Comparison of the average main effects of barberry shrub type, BAP hormone, IBA hormone on the investigated variables

Variable	Dry weight of leaves (gram)	Fresh weight of leaves(gram)	Leaf width to length ratio	Leaf surface (Mm)	Leaf length (Mm)	Leaf width (Mm)	Number of leaves	Germination (day)
BAP Hormone								
0	1.068 c	2.669 c	0.447 b	46.196 d	12.589 d	5.613 d	5.646 c	4.01 c
1	2.047 b	5.116 b	0.456 a	73.818 c	15.75 c	7.149 b	6.743 b	3.919 b
2	2.009 b	5.023 b	0.424 c	71.447 b	16.042 b	6.807 c	6.996 ab	3.71 b
3	2.376 a	5.94 a	0.446 b	81.06 a	16.703 a	7.428 a	7.248 a	3.396 a
Barberry type								
Seedless	1.858 b	4.643 b	0.429 c	64.643 b	15.125 b	6.464 c	6.965 a	4.158 b
Grainy	1.77 c	4.424 c	0.444 b	65.853 b	15 b	6.657 b	6.433 b	3.498 a
Ornamental	1.996 a	4.993 a	0.456 a	73.895 a	15.688 a	7.127 a	6.577 b	3.62 a
BAP Hormone								
0	1.512 b	3.78 b	0.447 a	59.756 b	14.287 b	6.353 b	6.253 b	4.032 b
0.25	2.238 a	5.593 a	0.44 b	76.504 a	16.255 a	7.146 a	7.063 a	3.485 a

Based on Duncan's multiple range test, numbers with the same letters in each column do not have significant differences.

Green time

The comparison of the average of the interaction effect between the type of IBA × BAP hormone showed that the lowest germination time was 3.374 days at a concentration of 3 mg l^{-1} of BAP hormone with a concentration of 0.25 mg l^{-1} of IBA hormone and the highest amount of germination time was 4.408 days at a concentration of 0 mg l^{-1} of BAP hormone with a concentration of Zero mg l^{-1} of IBA hormone was observed, although it did not show a statistical difference with some other treatments, but it was the lowest amount (Table 4).

Number of Leaves

The comparison of the mean of the interaction effect between barberry variety x IBA hormone showed that the highest number of leaves with 7.305 in the ornamental barberry variety with a concentration of 0.25 mg l^{-1} of the IBA hormone and the lowest number of leaves with 5.948 in the ornamental barberry variety with a concentration of 0 mg l^{-1} of the IBA hormone was observed. Although it did not show a statistical difference with some other treatments, it was the lowest value (Table 4).

Comparison of the mean of the interaction effect between the type of IBA × BAP hormone showed that the highest number of leaves with 7.761 numbers in the concentration of 3 mg l^{-1} of BAP hormone with the concentration of 0.25 mg l^{-1} of IBA hormone and the lowest number of leaves with 5.546 numbers in the concentration of zero mg l^{-1} of BAP hormone with zero mg l^{-1} concentration IBA hormone was observed, although it did not show a statistical difference with some other treatments, but it was the lowest amount (Table 4 and Figure 4).

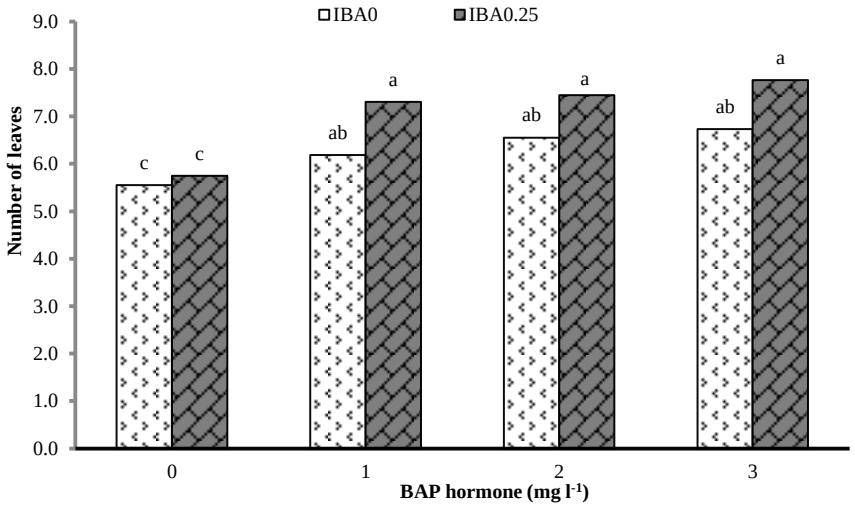

Figure 4. The interaction effect of IBA*BAP hormone on the number of leaves in barberry plant

Leaf length

The average comparison of the triple interaction effect between barberry type × BAP hormone × IBA hormone showed that the highest value of leaf length was 18.250 mm in the barberry type with a concentration of 0.25 mg l^{-1} of IBA hormone and 3 mg l^{-1} of BAP hormone, and the lowest value of leaf length was 11.500 mm in Barberry with a concentration of 0 mg l^{-1} of IBA and 0 mg l^{-1} of BAP was observed (Table 4).

Table 4. comparison of the average double interaction effect of barberry type*BAP hormone, barberry type*IBA and IBA*BAP variables under study

Interaction effects	Dry weight of leaves (gram)	Fresh weight of leaves(gram)	Leaf width to length ratio	leaf surface (Mm)	leaf length (Mm)	leaf width (Mm)	number of leaves	Germination (day)
Barberry type* BAP hormone								
BAP_0-seedless	0.995 c	2.486 c	0.449 ab	43.083 d	12.188 c	5.438 d	5.667 a	4.385 a
BAP_1-seedless	2.168 a	5.417 a	0.453 ab	73.519 ab	15.75 ab	7.131 ab	7.12 a	4.432 a
BAP_2-seedless	1.853 ab	4.632 ab	0.381 c	61.752 c	15.75 ab	6.013 c	7.505 a	4.109 a
BAP_3-seedless	2.417 a	6.039 a	0.435 ab	80.217 a	16.813 a	7.275 ab	7.568 a	3.703 a
BAP_0-grainy	1.039 c	2.596 c	0.429 b	46.18 d	12.844 c	5.513 cd	5.458 a	3.839 a
BAP_1-grainy	1.827 ab	4.564 ab	0.456 ab	69.13 bc	15.25 b	6.916 b	6.508 a	3.581 a
BAP_2-grainy	1.982 a	4.954 a	0.441 ab	69.174 bc	15.5 b	6.831 b	6.888 a	3.482 a
BAP_3-grainy	2.233 a	5.583 a	0.45 ab	78.927 ab	16.406 ab	7.369 ab	6.878 a	3.091 a
BAP_0-ornamental	1.169 bc	2.926 bc	0.464 a	49.326 d	12.734 c	5.888 cd	5.813 a	3.805 a
BAP_1-ornamental	2.147 a	5.366 a	0.458 ab	78.804 ab	16.25 ab	7.402 ab	6.603 a	3.743 a
BAP_2-ornamental	2.192 a	5.482 a	0.45 ab	83.416 a	16.875 a	7.578 a	6.595 a	3.538 a
BAP_3-ornamental	2.478 a	6.198 a	0.453 ab	84.035 a	16.891 a	7.641 a	7.298 a	3.395 a
Barberry type *IBA hormone								
IBA_0-seedless	1.472 b	3.679 b	0.421 c	55.452 c	14.25 b	5.938 d	6.625 ab	4.495 a
$IBA_{0.25}$-seedless	2.245 a	5.608 a	0.438 bc	73.833 b	16 a	6.991 b	7.305 a	3.82 a
IBA_0-grainy	1.533 b	3.83 b	0.453 ab	59.216 c	14.094 b	6.381 cd	6.188 ab	3.737 a
$IBA_{0.25}$-grainy	2.008 ab	5.018 ab	0.435 bc	72.49 b	15.906 a	6.933 b	6.678 ab	3.259 a
IBA_0-ornamental	1.532 b	3.832 b	0.466 a	64.601 bc	14.516 b	6.741 bc	5.948 b	3.863 a

$IBA_{0.25}$-ornamental	2.461 a	6.154 a	0.447 ab	83.189 a	16.859 a	7.513 a	7.206 a	3.377 a
IBA*BAP hormone								
IBA_0-BAP_0	0.898 f	2.243 f	0.45 ab	40.406 e	11.792 f	5.271 e	5.549 c	4.408 c
IBA_0-BAP_1	1.527de	3.817 de	0.466 a	61.036 c	14.208 d	6.601 c	6.184 ab	4.392 c
IBA_0-BAP_2	1.713 de	4.283 de	0.417 c	65.74 c	15.552 c	6.48 c	6.547 ab	3.908 b
IBA_0-BAP_3	1.911 cd	4.778 cd	0.454 ab	71.843 b	15.594 c	7.06 b	6.734 ab	3.418 a
$IBA_{0.25}$-BAP_0	1.238 ef	3.095 ef	0.445 ab	51.986 d	13.385 e	5.954 d	5.743 c	3.611 ab
$IBA_{0.25}$-BAP_1	2.567 ab	6.414 ab	0.446 ab	86.599 a	17.292 a	7.698 a	7.303 a	3.445 a
$IBA_{0.25}$-BAP_2	2.304 bc	5.762 bc	0.431 bc	77.154 b	16.531 b	7.134 b	7.445 a	3.511 ab
$IBA_{0.25}$-BAP_3	2.841 a	7.102 a	0.438 abc	90.277 a	17.813 a	7.796 a	7.761 a	3.374 a

Based on Duncan's multiple range test, numbers with the same letters in each column do not have significant differences.

Leaf width

Comparison of the average of the triple interaction effect between barberry type × BAP hormone × IBA hormone showed that the highest value of leaf width was 7.963 mm in ornamental barberry type with a concentration of 0.25 mg l^{-1} of IBA hormone and 3 mg l^{-1} of BAP hormone, and the lowest value of leaf width was 5.125 mm in the type of barberry. It was observed with a concentration of zero mg l^{-1} of IBA hormone and zero mg l^{-1} of BAP hormone (Table 5).

Leaf surface

The comparison of the average of the triple interaction effect between barberry type × BAP hormone × IBA hormone showed that the highest amount of leaf area with 93.151 square millimeters was in ornamental barberry type with a concentration of 0.25 mg l^{-1} of IBA hormone and 1 mg l^{-1} of BAP hormone and the lowest amount of leaf area was 38.045 square mm in the type of barberry. It was observed with a concentration of zero mg l^{-1} of IBA hormone and zero mg l^{-1} of BAP hormone (Table 5).

Leaf width to length ratio

The comparison of the average of the triple interaction effect between barberry type × BAP hormone × IBA hormone showed that the highest value of the leaf width to length ratio with 0.480 mm was in the ornamental barberry type with a concentration of zero mg l^{-1} of IBA hormone and 1 mg l^{-1} of BAP hormone, and the lowest value of the leaf width to length ratio with 0.480 mm 0.337 mm was observed in the type of barberry with a concentration of zero mg l^{-1} of IBA hormone and 1 mg l^{-1} of BAP hormone (Table 5).

Fresh weight of leaves

The average comparison of the triple interaction effect between barberry variety x BAP hormone x IBA hormone showed that the highest amount of leaf fresh weight with 7.532 grams was in the type of barberry with a concentration of 0.25 mg l^{-1} of IBA hormone and 3 mg l^{-1} of BAP hormone and the lowest amount of leaf fresh weight with 2.220 grams in Barberry with a concentration of 0 mg l^{-1} of IBA and 0 mg l^{-1} of BAP was observed (Table 5).

Leaf dry weight

The average comparison of the triple interaction effect between barberry type × BAP hormone × IBA hormone showed that the highest amount of leaf dry weight was 3.013 grams in the type of barberry with a concentration of 0.25 mg l^{-1} of IBA hormone and 3 mg l^{-1} of BAP hormone, and the lowest amount of leaf dry weight was 0.888 grams in Barberry with a concentration of 0 mg l^{-1} of IBA and 0 mg l^{-1} of BAP was observed (Table 5).

Table 5. Comparison of the triple interaction effect of barberry type*BAP hormone*IBA hormone on the studied variables

Interaction effects	Dry weight of leaves	Fresh weight of leaves(gram)	Leaf width to length ratio	leaf surface (Mm)	leaf length (Mm)	leaf width (Mm)
IBA0-BPA0-seedless	0.888 g	2.22 g	0.451 ab	38.045 g	11.5 e	5.125 d
IBA0-BPA1-seedless	1.692 d-g	4.23 d-g	0.457 ab	62.632 d	14.5 bc	6.638 ab
IBA0-BPA2-seedless	1.487 e-g	3.718 e-g	0.337 c	53.447 e	15.625 b	5.263 c
IBA0-BPA3-seedless	1.82 c-g	4.547 c-g	0.44 ab	67.683 bc	15.375 b	6.725 ab
IBA0.25-BPA0-seedless	1.102 fg	2.752 fg	0.447 ab	48.12 f	12.875 d	5.75 bc
IBA0.25-BPA1-seedless	2.643 a-d	6.603 a-d	0.448 ab	84.405 ab	17 ab	7.625 a
IBA0.25-BPA2-seedless	2.22 a-e	5.545 a-e	0.426 b	70.057 c	15.875 b	6.763 ab
IBA0.25-BPA3-seedless	3.013 a	7.532 a	0.429 b	92.751 a	18.25 a	7.825 a
IBA0-BPA0-grainy	0.918 g	2.292 g	0.429 b	41.823 g	12.25 d	5.25 c
IBA0-BPA1-grainy	1.452 efg	3.628 efg	0.459 ab	56.018 d	13.75 c	6.269 b
IBA0-BPA2-grainy	1.877 b-g	4.687 b-g	0.453 ab	68.35 c	15.188 b	6.869 ab
IBA0-BPA3-grainy	1.885 b-g	4.715 b-g	0.469 ab	70.672 c	15.188 b	7.138 ab
IBA0.25-BPA0-grainy	1.16 efg	2.9 efg	0.429 b	50.537 ef	13.438 c	5.775 bc
IBA0.25-BPA1-grainy	2.202 a-e	5.5 a-e	0.452 ab	82.242 b	16.75 ab	7.563 a
IBA0.25-BPA2-grainy	2.087 a-f	5.222 a-f	0.429 b	69.998 bc	15.813 b	6.794 ab
IBA0.25-BPA3-grainy	2.582 a-d	6.45 a-d	0.431 b	87.183 ab	17.625 ab	7.6 a
IBA0-BPA0-ornamental	0.887 g	2.218 g	0.47 a	41.35 g	11.625 e	5.438 c
IBA0-BPA1-ornamental	1.437 efg	3.593 efg	0.48 a	64.457 cd	14.375 bc	6.897 ab
IBA0-BPA2-ornamental	1.777 d-g	4.443 defg	0.461 ab	75.424 bc	15.844 b	7.309 ab
IBA0-BPA3-ornamental	2.028 a-f	5.072 a-f	0.452 ab	77.172 b	16.219 b	7.319 ab
IBA0.25-BPA0-ornamental	1.452 e-g	3.633 e-g	0.457 ab	57.301 d	13.844 c	6.338 b
IBA0.25-BPA1-ornamental	2.857 a-c	7.138 a-c	0.437 b	93.151 a	18.125 a	7.906 a
IBA0.25-BPA2-ornamental	2.607 a-d	6.52 a-d	0.438 b	91.408 a	17.906 a	7.847 a
IBA0.25-BPA3-ornamental	2.928 ab	7.323 ab	0.454 ab	90.897 a	17.563 a	7.963 a

Based on Duncan's multiple range test, numbers with the same letters in each column do not have significant differences.

DISCUSSION

Also, the results showed that with the increase in the length and width of the leaf, the surface of the leaf increases. Which is consistent with Nazari [21] results. Also, Falahi [22] reported that increasing the length of the leaf blade increases the leaf surface and increases the photosynthetic activity.

According to the results of the research conducted in barberry, it has been stated that there may be a genetic defect related to the reaction to rooting stimuli in this plant. In this regard, many systems have been proposed to create genetic defects in different plants [21]. In this research, perhaps one of the reasons for the lack of rooting is the aforementioned reason. In other researches, it has been stated that in cuttings of barberry, callus is produced under the skin, which is associated with swelling and finally splitting of the skin, but this Callus formation does not cause roots, or very fragile and weak roots are formed and are easily separated from the trunk.

Mohammadi *et al.*, stated that in relation to stem tissue culture, the problem of not forming roots is very severe and still remains because the cutting site in the samples that turn brown, callus induction is not observed and eventually they disappear. Similar results were obtained in this research. But root production was observed in ornamental and seed barberry [27].

In an experiment, Mazeginejad investigated the in vitro viability of Berberis vulgaris L. calluses. and in the first experiment, the combination of hormones suitable for the induction and growth of callus from barberry leaves and

environment (MS/2+ 0.5 mg l^{-1} 2-4-D + 2 mg l^{-1} BAP) He reported the best combination of hormones. In another experiment to determine the optimal culture medium for the survival of the two hormones BAP (2 mg) and TDZ (15 mM) separately in combination with each of the factors methyl jasmonate (1 and 2 mg), iron oxide nanoparticles (75 and 100 ppm) and used zinc oxide nanoparticles (75 and 100 ppm). Finally, the external observation of callus viability showed that treatments containing BAP had higher viability than TDZ treatments in terms of appearance. So that in the treatment of BAP and Nano zinc oxide with a concentration (75 ppm) 100% survival was reported. In general, the evaluation of calluses reported the high performance of nanoparticles, especially zinc oxide nanoparticles, on the reduction of phenolic compounds and browning [19].

In a research, Jain and Basheer reported the concentration of 1 mg l^{-1} TDZ along with 0.1 mg l^{-1} IBA as the best regeneration treatment after 28 days, then in order to continue growing and multiplying, the branches in MS medium containing 0.1 mg l^{-1} of IBA plus 0.5 mg l^{-1} of gibberellic acid and reported [23]. Therefore, in many cases, the results of this research were consistent with the results of other researchers.

The correlation coefficient (Table 6) showed that between leaf width and length (r=0.83**), between leaf length and surface (r=0.95**), between width and surface area (r=0.96**), between leaf fresh weight with the number of leaves (r=0.87**), leaf length (r=0.72**), leaf width (r=0.68**), leaf surface (r=0.74**), and between the dry weight of leaves with the number of leaves (r=0.88**) and leaf length (r=0.74**), leaf width (r=0.74**) and leaf surface (r=0.75**) were positive and significant at 1% probability level. Nazari *et al* stated that there was a positive and significant correlation between leaf width and leaf area at the probability level of 1% [24]. Which is consistent with the results of this research. Considering that in this research, seed germination in seeded barberry were also investigated, which were very successful, so as a solution to the problem of barberry propagation, seeded barberry seeds can be used for propagation and then, by transplanting barberry on seeded barberry bases, the began mass production of barberry (Figure 5- 7).

Table 6. Correlation coefficient between investigated traits in barberry shrub under glass conditions under the influence of shrub type and different amounts of BAP*IBA hormone

variable	Time to turn green	number of leaves	leaf width	leaf length	Leaf surface	Leaf width to length ratio	Fresh weight of leaves	Germination
Germination	1.00							
number of leaves	0.12	1.00						
leaf width	.400**	.367**	1.00					
leaf length	.42**	.33**	.83**	1.00				
leaf surface	.42**	.36**	.95**	.96**	1.00			
Leaf width to length ratio	-0.05	0.17	-.23**	.35**	0.07	1.00		
Fresh weight of leaves	-0.10	.87**	.72**	.68**	.74**	0.10	1.00	
Dry weight of leaves	-0.10	.88**	.74**	.74**	.75**	0.16	0.96**	1.00

ns, * and ** are not significant, significant at 5% and 1% level, respectively.

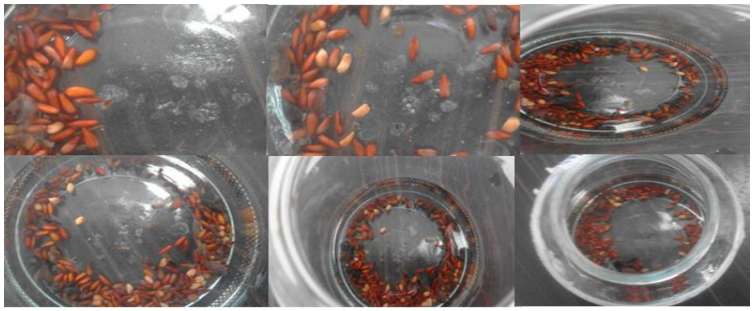

Figure 5. Place grainy barberry seeds for germination

Figure 6. Placement of grainy barberry seed in tissue culture medium

Figure 7. Greening of grainy barberry and continued growth in natural conditions

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Conflict of interests

NO conflict

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