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IN VITRO ORGANOGENESIS AND MICROPROPAGATION OF *Ornithogalum sigmoideum*

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Abstract: *Ornithogalum sigmoideum* L., is a common species in Anatolia, grows in gardens in the Black Sea region and is consumed as a vegetable. *Ornithogalum sigmoideum* L. bulbs can be used in many areas such as commercial production, ornamental plants and secondary metabolites for medicine. In the study, the bulbs of *Ornithogalum sigmoideum* is one of the geophytes of Türkiye, were used as genetic material and were cultured in *in vitro*. The objective of the study was to investigate of Kinetin (Kn) and 6-Benzylaminopurine (BAP) as plant growth regulators and combination with 0.2 mg L^{-1} NAA (α-naphthalene acetic acid) and 0.2 mg L^{-1} GA_3 (Gibberellic acid) for whole plant regeneration and bulb from shoot cultured of *Ornithogalum sigmoideum* L. Supporting of Kn at 2.0 mg L^{-1} concentration had the highest shoot number as 7.0. Similarly, BAP combination with NAA promoted direct adventitious shoot proliferation. On the other hand, different concentrations of indole-3-butyric acid (IBA) considerably improved root formation for full plant. Media of 1.0 mg L^{-1} IBA had the highest mean in terms of root number (4.0) and root length (3.5 cm). 2.0 mg L^{-1} IBA had the highest mean for shoot length with 5.1 cm.

Keywords: Bulbs, *In vitro* regeneration, Shooting, Rooting

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1. Introduction

Natural flower bulbs, called geophytes, continue their lives underground with storage organs such as onions, corms, bulbs, tubers and rhizomes (Altan, 1985; Aksu et al., 2002; Avci, 2005; Kahraman and Ozzambak 2015; Demir Percin et al., 2026). Türkiye has a very rich geophyte species with 1,056 taxa bulbous, rhizome and tuberous (Ozhatay, 2013). *Ornithogalum sigmoideum* is a naturally grown species in Türkiye's geophytes (Yalcin et al., 2020; Atak et al., 2026). It is also known such as “ak yildiz”, “saliva grass” and “dog onion”. The mucilage contained in its onions is used as a thickener and is known as an emetic and a decongestant. It also strengthens the contraction of the heart. In addition, bulbs contain large amounts of needle-shaped crystals and small amounts of starch in the form of small grains (18-28 microns in diameter) (Heves, 2008; Kaynak, 2017). Ak yildiz bulbs can be an alternative to synthetic drugs, especially in the field of medicine, and seconder metabolite its natural extracts can be used eye infection in the treatment of *Acanthamoeba amoeba* (Kaynak, 2017; Demir Percin et al., 2026). *O. sigmoideum*, commonly known as the "pregnant onion" is difficult to propagate due to its slow growth rate and the impracticality of seed propagation (Malabadi et al., 2004). From a single mother bulb, about 4 to 6 bulbs will be produced in a year. On the other hand, the rate of growth of the bulbs is very poor in one year (Naik and Nayak, 2005). Seed propagation is not practical either. It takes at least 4 to 5 years from seed to flowering. This lengthy process makes seed propagation inefficient for *O. sigmoideum* (Wabule et al., 1991). So *in vitro* propagation is an alternative method for the multiplication of bulbous for *O. sigmoideum*. In this case, tissue culture production is suggested as an advantage over traditional production. It allows for rapid propagation and disease free bulbs. Studies have shown its effectiveness in producing healthy plantlets for cultivation purposes, thereby addressing the challenges associated with slow growth rates and limited multiplication through traditional means (Kim et al., 1996). With tissue culture, *in vitro* production can be made in bulbous plants, as in other cultivated plants. In this regard, tissue culture studies have been carried out mostly on geophytes such as narcissus, tulips and snowdrops (Sesiz, 2014). Therefore, *O. sigmoideum* could be multiplied rapidly and lots at the same time (Debergh and Zimmerman, 1991; Hartman et al., 1997; Ozturk, 2021a; Ozturk, 2022). On the other hand, in micropropagation, various factors including the type of explant used, culture conditions, composition of the culture medium, and concentration of growth regulators play crucial roles in the success and efficiency of bulb regeneration (Krikorian and Kann, 1986; Ziv and Lilien-Kipnis, 1997; Kumar et al., 2002; Ozturk, 2021b).

Micropropagation studies have been carried out in several species of *Ornithogalum* (Rensburg et al., 1989; Nayak and Sen, 1995a; Ziv and Lilien-Kipnes, 2000; Kariuki and Kako, 2003; Malabadi et al., 2004; Naik and Nayak, 2005; Suh et al., 2005; Ozel and Khawar, 2007; Ozel et al., 2008; López-Marín et al., 2009; Karaguzel et al., 2012; Joung et al., 2020; Petti, 2025). Although there have been many studies on the *in vitro* regeneration of *Ornithogalum* species, studies on *O. sigmoideum* are relatively limited compared to other ornamental plants or geophytic taxa. As one of the prominent geophytes in Türkiye, *O. sigmoideum* demonstrates strong potential for tissue culture-based propagation. This approach offers a significant advantage by enabling the rapid, large-scale, and disease-free production of planting material, thereby accelerating its commercial utilization.

This study aimed to obtain a full plant by *in vitro* regeneration and rooting of natural bulbs of *O. sigmoideum*, which is among the geophytes that have a wide production area in nature in Türkiye. Also analyzed the suitability of different concentrations Kn, BAP, NAA and IBA in the culture medium for efficient regeneration.

2. Materials and Methods

2.1. Source of the Explant

The study was carried out in Tissue Culture Laboratory of the Field Crops Department of Agricultural Faculty of the Ege University. Natural *O. sigmoideum* bulbs collected from 1600 m high mountain villages of the Black Sea Region were used as genetic material in the study.

2.2. Promotion of Adventitious Buds

O. sigmoideum bulbs were cultured *in vitro* on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). The hormone-free MS medium was used as the control and designated as MS0. MS+ 0.2 mg L^{-1} NAA+0.2 mg L^{-1} GA_3 containing Kn 0.5 mg L^{-1} (MS1); 1.0 mg L^{-1} (MS2); 2.0 mg L^{-1} (MS3); 3.0 mg L^{-1} (MS4) and BAP 0.5 mg L^{-1} (MS5); 1.0 mg L^{-1} (MS6); 2.0 mg L^{-1} (MS7); 3.0 mg L^{-1} (MS8) were evaluated as multiplication media with 0.6 % agar-agar (MERCK, Germany). All media were prepared with 3% (w/v) sucrose and the pH value was 5.8. Before surface disinfection, *O. sigmoideum* bulbs were washed in tap water, cleaned, and kept in bleach for 30 minutes. Finally, they were kept in distilled water until the culture stage. For surface disinfection, bulbs were kept in 70% ethanol and 2.5% sodium hypochlorite (NaOCl) for 10 minutes, then washed 3-4 times with distilled water. After surface sterilization, basal parts of each bulb were divided into 4 parts from the basal axis and *in vitro* cultured. The *in vitro* regeneration of *O. sigmoideum* bulbs was achieved after six weeks, and the parameters of shoot number, shoot length, root number, and root length were evaluated. The experiment was carried with one explant per tube in the Completely Randomized Plot Design with 2 replications. A total of 180 explants, 10 for each nutrient medium, were cultured. They were cultured at 23±2 °C for 16 hours of white fluorescent light (30 µmol $m^{-2}s^{-1}$) for their growth.

2.3. Promotion of Roots

Microbulbs of approximately 2-4 cm-long were stimulated to root on MS supplemented with different concentrations of IBA 0 mg L^{-1} (R1); 0.1 mg L^{-1} (R2); 0.5 mg L^{-1} (R3); 1.0 mg L^{-1} (R4); 2.0 mg L^{-1} (R5). The media were fortified with 2% sucrose (w/v) and gelled with 0.6% agar (MERCK, Germany). The pH was adjusted to 5.8. The cultures were grown at 25±1 °C under illumination for 16 hours photoperiod with a light intensity of 50 - 60 µmol $m^{-2}s^{-1}$. The regenerated shoots were cultured in Completely Randomized Plot Design with 3 replications. The data obtained for shoot and root were analyzed by the standard technique of Analyses of Variance (ANOVA) and the means were compared by using the Least Significant Difference (LSD) test as described by Steel and Torrie (1980).

3. Results and Discussion

3.1. Effect of PGR on Shoot Organogenesis from Bulbs Explants

Shoot and root regenerations of bulbs of *O. sigmoideum* were investigated *in vitro* in this study. Shoot regeneration was evaluated in nine different culture media (Table 1).

Table 1. Means of shoot number, shoot length (cm), root number, root length (cm) and F values in different nutrient media for *Ornithogalum sigmoideum* bulb explants

Number of medium	Treatment	Shoots number	Shoots length (cm)	Root number	Root length (cm)
MS0	MS (Control)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MS1	0.5 mg L^{-1} Kn	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MS2	1.0 mg L^{-1} Kn	2.5 ± 0.41^{bcd}	1.0 ± 0.0 ^{de}	1.3 ± 0.19^{c}	2.4 ± 0.04^{a}
MS3	2.0 mg L^{-1} Kn	7.0 ± 0.07^{a}	2.7 ± 0.09^{b}	2.5 ± 0.04^{b}	1.3 ± 0.08^{b}
MS4	3.0 mg L^{-1} Kn	4.0 ± 0.07^{b}	2.0 ± 0.32^{bc}	0.0 ± 0.0	0.0 ± 0.0
MS5	0.5 mg L^{-1} BAP	3.0 ± 0.50^{bc}	7.6 ± 0.04^{a}	4.0 ± 0.08^{a}	2.6 ± 0.34^{a}
MS6	1.0 mg L^{-1} BAP	2.0 ± 0.32^{cd}	1.7 ± 0.26^{cd}	3.5 ± 0.04^{a}	1.5 ± 0.22^{b}
MS7	2.0 mg L^{-1} BAP	4.0 ± 0.68^{b}	1.4 ± 0.20^{cd}	0.5 ± 0.07^{cd}	0.5 ± 0.14^{c}
MS8	3.0 mg L^{-1} BAP	1.0 ± 0.17^{de}	0.5 ± 0.07^{ef}	0.0 ± 0.0	0.0 ± 0.0
$LSD_{(0.05)}$		1.599	0.856	0.961	0.729
F		19.944**	75.600**	29.038**	21.170**

**: significant at the 0.01 probability level

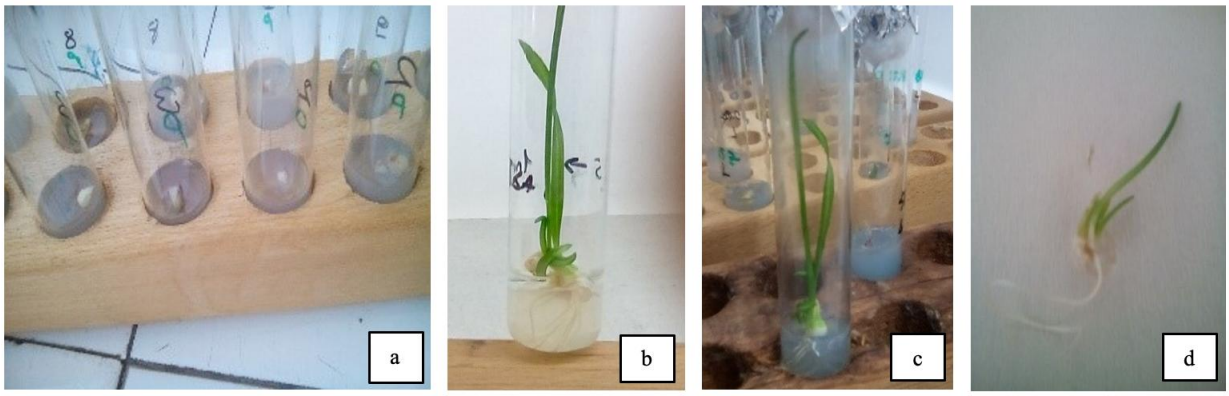

Figure 1. *In vitro* culture stages of *Ornithogalum sigmoideum* bulbous (a) *Initial culture of O. sigmoideum bulbs* b-c) Development of rooted plantlets and obtaining complete plants d) Acclimatization of regenerated plants before transfer to pots).

Statistical differences at the p≤0.01 significance level were observed in all evaluated parameters (Table 1). In terms of shoot number, the highest mean was obtained by MS3 medium (2.0 mg L^{-1} of Kn) with an average of 7.0, followed by media containing 3.0 mg L^{-1} Kn (MS4) and 2.0 mg L^{-1} BAP (MS5) with 4.0. In terms of shoot length, the medium containing MS+0.5 mg L^{-1} BAP (MS5) was found to be the highest with 7.6 cm. On the other hand, the cultured explants showed any proliferation in 0.5 mg L^{-1} Kn and MS (no plant growth regulator-MS0) (Table 1). Table 1 also shows the rooting results obtained at this stage, although the main aspect studied was the ability of the initial explants to multiply and develop shoots. The highest mean was obtained from 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} BAP as 4.0 and 3.5, respectively for root number. Root length had the highest mean in media containing 1.0 mg of Kn and 0.5 mg of BAP for per liter with 2.4 cm and 2.6 cm, respectively.

Figure 1 shows pictures of the various stages of the experiment. Different studies have been conducted on the *in vitro* regeneration of *Ornithogalum* species, in order to evaluate the effects of nutrient media components, culture conditions and different explants were investigated (Rice et al., 1983; Krikorian and Kann, 1986; Nayak and Sen, 1995b; Ziv and Lilien-Kipnis, 2000; Malabadi et al., 2004; Ozel and Khawar, 2007; López-Marín et al., 2009). Many authors emphasized that the regeneration of bulb scales was high in the regeneration of *Ornithogalum* species, and media containing different concentrations of NAA as an auxin, and BA (6-Benzyladenine), BAP (6-Benzylaminopurine), and Kinetin as cytokinin were suitable (Suh et al., 2005; Naik and Nayak, 2005; Ozel et al., 2008; Karaguzel et al., 2012). Our study showed that BAP and Kn were used as cytokinins and both plant growth regulators were effective in shoot regeneration. It was determined that the number of shoots of O. arabicum species was the highest when regenerated in liquid medium containing 0.01 mg L^{-1} NAA and 5.0 mg L^{-1} BA (Yanagawa and Ito, 1988).

Shoots were obtained from callus in medium containing 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} NAA (Nayak and Sen, 1995b). Our study indicated that BAP combined auxin (NAA) and GA_3 was more potent than Kn combined auxin (NAA) and GA_3 in direct shoot regeneration without callus formation. Naik and Nayak (2005) reported that in the *in vitro* regeneration of *O. virens*, two stages followed each other in the formation of new bulbs in different nutrient media. The first phase of callus growth at the base is followed by another phase involving differentiation and regeneration. Medium of 1.0 mg L^{-1} NAA and 2.0 mg L^{-1} BA and 60 g/L sucrose were found to be suitable for direct bulb formation on scaly leaves. High concentrations of cytokines reduce callus formation and shoot bud induction (Tiwari et al., 2001). Generally, higher concentrations of increased shoot formation, total shoot number, and shoot length, depending on the type of PGR and explant types. Similarly, a high percentage of bulbs per explant (4.97) was obtained in the medium containing 1.5 mg L^{-1} BA + 0.50 mg L^{-1} NAA or 4 mg/ L^{-1} BAP + 0.50 mg L^{-1} NAA (Suh et al., 2005; Karaguzel et al., 2012). Ozel et al. (2008) reported that 4.83 shoots were obtained in the medium containing 2.0 mg L^{-1} BAP + 0.50 mg L^{-1} NAA. The results obtained point to the great regenerative capacity of these *Ornithogalum* species and new bulb growth. López-Marín et al. (2009) reported that nutrient media containing 1.5 mg L^{-1} BAP were suitable for the regeneration of different explant sources (leaves, inflorescences, bulbs) of different *Ornithogalum* species *in vitro* conditions. It was also emphasized that contamination prevents culture development after the regeneration of bulb explants. The presence of fungal and bacterial contamination risk increased and adversely affected shoot growth (Ziv and Lilien-Kipnis, 2000; López-Marín et al., 2009; Karaguzel et al., 2012). In this study, among the different Kn and BAP concentrations tested in the presence of 0.2 mg L^{-1} NAA and 0.2 mg L^{-1} GA_3, it was determined that 2 mg L^{-1} Kn was effective on shoot number, 0.5 mg L^{-1} BAP was found to be the most suitable for shoot length, root number, and root length in *O. sigmoideum.*

3.2. Effect of PGR on Rooting from Shoot

Shoots raised on BAP supplemented media when transferred to IBA medium exhibited high rooting for whole plant regeneration. The results of root regeneration from shoots of *O. sigmoideum* are presented (Table 2).

Table 2 presents that all evaluated parameters for number of plantlets, plantlet length (cm), number of roots, and root length (cm) showed statistically significant differences at the P≤0.01 level. In terms of plantlet number, the highest mean (5.7) was obtained from the 1.0 mg L^{-1} IBA medium, followed by the 2.0 mg L^{-1} IBA medium with 4.3. For plantlet length, the MS + 2.0 mg L^{-1} IBA medium recorded the highest mean with 5.1 cm. The highest root number was observed in the medium containing 1.0 mg L^{-1} IBA with a mean of 4.0. Regarding root length, the highest values were obtained from the media containing 0.5 mg L^{-1} IBA (3.7 cm) and 1.0 mg L^{-1} IBA (3.5 cm), respectively. The analysis of rooting percentage clearly demonstrates the influence of IBA concentration on the *in vitro* rooting efficiency of *O. sigmoideum*. It was reported that NAA hormone, an auxin, was effective for rooting at a concentration of 0.5 to 5 µM (Kariuki and Kako, 2003). It was reported that, during the in vitro regeneration of *O. saundersiae* bulbs, shoot development was partially achieved in MS + TDZ, whereas shoot and root formation occurred in MS + BAP + NAA (Hutchinson et al., 2004). In our study, media in which Kn and BAP were combined with NAA+ GA_3 were used. Then subculture processes were carried out for the root development of the developing shoots. In the case of *O. sigmoideum*, 1.0 mg L^{-1} IBA is the best auxin for rooting from bulblet. These results collectively indicate that moderate concentrations of IBA, particularly 1.0 mg L^{-1}, are optimal for achieving high rooting success in *O. sigmoideum* under *in vitro* conditions.

Table 2. Means of shoot number, shoot length (cm), root number, root length (cm) and F values in different nutrient media for *Ornithogalum sigmoideum* shoots explants

Number of medium	Treatment	Shoots number	Shoots length (cm)	Root number	Root length (cm)
R1	MS (Control)	1.0 ± 0.13^{d}	1.6 ± 0.24^{c}	0.0 ± 0.0	0.0 ± 0.0
R2	0.1 mg L^{-1} IBA	2.4 ± 0.39^{c}	4.0 ± 0.48^{b}	1.40 ± 0.20^{c}	1.6 ± 0.24^{b}
R3	0.5 mg L^{-1} IBA	3.9 ± 0.08^{b}	4.0 ± 0.12^{b}	2.7 ± 0.45^{b}	3.7 ± 0.30^{a}
R4	1.0 mg L^{-1} IBA	5.7 ± 0.17^{a}	3.2 ± 0.38^{b}	4.0 ± 0.10^{a}	3.5 ± 0.13^{a}
R5	2.0 mg L^{-1} IBA	4.3 ± 0.41^{b}	5.1 ± 0.35^{a}	0.4 ± 0.01^{d}	0.5 ± 0.14^{c}
$LSD_{(0.05)}$		1.240	0.908	0.488	0.760
F		20.826**	20.820**	112.810**	49.252**

**: significant at the 0.01 probability level

4. Conclusion

This study successfully established a simple and efficient protocol for shoot regeneration through direct adventitious bulb formation. The results showed that the combination of auxin (NAA) and GA_3 with BAP was more effective than NAA and GA_3 with Kn for *in vitro* direct shoot regeneration from natural bulbs of *Ornithogalum sigmoideum*, highlighting the high regenerative capacity of this species under optimized culture conditions. Moreover, refinement of the protocol by transferring bulbs into rooting media containing different concentrations of IBA further improved root induction and plantlet development.

The regeneration system optimized in this study provides a rapid and reliable method for *mass micropropagation* of *O. sigmoideum*. Future research should aim to commercial propagation the protocol also constitutes a valuable strategy for the conservation of *O. sigmoideum* germplasm. The aim here was to obtain full plants with a good root formation of the shoots. For that reason, the plantlets obtained *in vitro* conditions could have a great advantage in adapting in outdoor.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	M.A.A.	G.O.
C	50	50
D	50	50
S	40	60
DCP	60	40
DAI	40	60
L	60	40
W	50	50
CR	40	60
SR	50	50
PM	40	60
FA		100

C=n concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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