ROOTING AND ACCLIMATIZATION OF *IN VITRO* MICROPROPAGATED SNOWDROP (*Galanthus ikariae* BAKER.) BULBLETS*

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Abstract

Rooting and acclimatization phases of *Galanthus ikariae* micropropagation were studied using bulblets produced *in vitro* from bulb-scales. For rooting, bulblets were cultured on full, 1/2, 1/4 or 1/8 strength modified Murashige and Skoog (MMS) media which contained different concentrations of sucrose (30, 60 $g \cdot L^{-1}$), activated charcoal (AC) (0.2, 0.5 and 1.0%) and naphthalene acetic acid (NAA) (0, 0.01, 0.1 and 0.5 $mg \cdot L^{-1}$). Cultures were incubated in a growth chamber at 23°C, 16 / 8 hour photoperiod for 16 week. The results indicated that NAA concentration is important factor for root formation. The effects of other factors such as medium strength, AC and sucrose concentrations on rooting were not found statistically signifficant. Compared to other dosages of NAA, the highest rate of rooting was obtained from culture media containing 0.5 $mg \cdot L^{-1}$ NAA. For the transplantation stages of experiments, rooted bulblets in 1/8 MMS medium which contained 0.5% AC, 0.5 $mg \cdot L^{-1}$ NAA and 60 $g \cdot L^{-1}$ sucrose concentration and 1/2 MMS medium which contained 0.5% AC, 0.5 $mg \cdot L^{-1}$ NAA and 30 $g \cdot L^{-1}$ sucrose concentration were transferred either directly to the sterile soil mixture or incubated for 8 weeks at 4°C or incubated for 4 weeks in MMS medium contained 2% sucrose and different concentrations of gibberellic acid (GA_3) (10 or 50 $mg \cdot L^{-1}$) and then to soil. Although the differences between the proportions obtained from treatments were statistically unsignificant, the highest rate of survival was determined as 28 % in bulblets which were rooted in 1/2 MMS medium containing 30 $g \cdot L^{-1}$ sucrose, 0.5 % AC and 0.5 $mg \cdot L^{-1}$ NAA and then transferred directly to the soil.

Keywords: *Galanthus,* micropropagation, rooting, acclimatization

İn vitro Olarak Mikro Çoğaltımı Yapılmış Kardelen (*Galanthus ikariae* Baker.) Soğancıklarının Köklendirilmesi ve Dış Koşullarda Geliştirilmesi

Özet

Galanthus ikariae' nin soğan pul yapraklarından *in vitro* olarak elde edilen adventif soğancıkların köklendirilmesi ve dış koşullara aktarma aşaması çalışılmıştır. Köklendirme için, soğancıklar farklı konsantrasyonlarda sukroz (30, 60 $g \cdot L^{-1}$), aktif kömür (AK) (% 0.2, 0.5 ve 1.0), naftalen asetik asit (NAA) (0, 0.01, 0.1 ve 0.5 $mg \cdot L^{-1}$) içeren tam, 1/2, 1/4, 1/8 kuvvette hazırlanmış değiştirilmiş Murashige and Skoog (DMS) ortamlarında kültüre alınmıştır. Kültürler 23°C sıcaklık ve 16/8 saat aydınlık/karanlık fotoperyoduna ayarlanmış iklim dolabında 16 hafta süreyle inkübe edilmiştir. Sonuçlar, NAA konsantrasyonun kök oluşumu için önemli faktör olduğunu göstermiştir. Diğer faktörler; Ortam kuvveti, AK ve sukroz konsantrasyonlarının köklenme üzerindeki etkileri istatistiksel olarak önemli bulunmamıştır. En yüksek köklenme oranı 0.5 $mg \cdot L^{-1}$ NAA içeren kültür ortamlarında elde edilmiştir. Dış koşullara aktarma aşaması denemeleri için 1/8 DMS, 0.5 $mg \cdot L^{-1}$ NAA, % 0.5 aktif kömür ve 60 $g \cdot L^{-1}$ sukroz içeren ortam ile 1/2 DMS, 0.5 NAA, % 0.5 aktif kömür ve 30 $g \cdot L^{-1}$ sukroz içeren ortamlarada köklendirilmiş soğancıklar ya direkt olarak veya 8 hafta 4°C' de inkube edildikten sonra veya %2 sukroz ve gibberelik asittin (GA_3) değişik dozlarını (10 ve 50 $mg \cdot L^{-1}$) içeren DMS ortamında 4 hafta inkübe edildikten sonra toprağa aktarılmışlardır. Uygulamalardan elde edilen oranlar arasındaki farklar istatistiksel olarak önemsiz olmasına rağmen bu uygulamalar içinde en yüksek yaşama oranı 1/2 DMS, 0.5 NAA, % 0.5 aktif kömür ve 30 $g \cdot L^{-1}$ sukroz içeren ortamda kültüre alınarak köklendirilen ve direkt toprağa aktarılan soğancıklarda görülmüş ve % 28 olarak belirlenmiştir.

Anahtar Kelimeler: *Galanthus*, Micro Çoğaltım, Köklenme, Dış Koşullara Alıştırma.

1.Introduction

The rate of natural vegetative propagation of *Galanthus* is very slow. In addition to the long propagation period of this species, the overharvesting decreased *Galanthus* stock to dangerous levels. Ekim et al. (2000) pointed out that varieties in this species were categorized as vulnerable. Currently, there is considerable need to develop methods, which shorten the propagation period under controlled

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conditions. Propagation through tissue culture has been used successfully in various plant species and geopyhtes also. But there is limited research on the propagation of *Galanthus* species through tissue culture (Popov and Cherkasov, 1984; Girmen, 1986). In these research studies, *in vitro* propagation of different *Galanthus* species bulblets was carried out, but there is no information on the rooting and transplantation stages. When it is aimed to propagate with tissue culture, one of the important stages is providing for the transformation of the tissue piece to a complete plant by rooting the vegetative propagation material such as the shoots or bulblets that are formed.

The first studies related to the propagation of *G. ikariae* by tissue culture were done by our research group in previous years (Tıpırdamaz et al., 1999). In these researches, first stage covering *in vitro* propagation of bulblets of *G. ikariae* was completed. In the present study, the main objective was to investigate the effect of medium constituents (NAA, AC and sucrose concentrations and medium strength) on root formation of *G. ikariae* bulblets produced *in vitro*. The transplantation stage to *in vivo* conditions was also studied.

2. Material and methods

Bulbs of *G. ikariae* were obtained from naturally grown environment. Sterilization of materials, preparation of explants and culture medium, production of *in vitro* bulblets were done according to Tıpırdamaz et al. (1999). For the sterilization of bulb tissues, bulbs were prewashed with water, held in 96 % ethyl alcohol for 3 minutes, sterilized in 40% commercial sodium hypochlorite (NaOCl) solution with added Tween 20 for 20 minutes and washed with sterile distilled water 3 times for 5 minutes. Bulb scales consisting of pieces 5 mm wide, 8-10 mm high including 2 mm basal plate were used as explant source. The basic nutrient medium consisted of the salt mixture of Murashige and Skoog (1962), the levels of organic substances were determined according to Girmen (1986); 100 $mg \cdot L^{-1}$ *myo*-inositol, 0.5 $mg \cdot L^{-1}$ thiamin HCl, 0.5 $mg \cdot L^{-1}$ pyridoxine-HCl, 2.0 $mg \cdot L^{-1}$ glycine, 1.0 $mg \cdot L^{-1}$ nicotinic acid. This medium was designated Modified Murashige and Skoog (MMS) medium. Bulb scales were cultured on MMS medium supplemented with 6% sucrose, 0.2 $mg \cdot L^{-1}$ potassium salt of naphthalaneacetic acid (KNAA) + 2.0 $mg \cdot L^{-1}$ BAP and %0.6 agar as described by Tıpırdamaz et al. (1999) and the pH was adjusted to 5.5. Cultures were maintained in a growth chamber at 20°C with a 16 h photoperiod provided by fluorescent and incandescent lights (2000 lux) for 16 weeks. Produced bulblets were transferred to MMS medium of full, 1/2, 1/4 and 1/8 strenght containing different concentrations of sucrose (30 or 60 $g \cdot L^{-1}$), AC (0.2, 0.5 and 1.0%) and NAA (0, 0.01, 0.1 and 0.5 $mg \cdot L^{-1}$) at pH 5.5. Explants or produced bulblets above mentioned were cultured in 100 x 20 mm test tubes containing 10 ml MMS medium. Each test tube had one explant. 50 tubes were used for each treatment. The cultures were incubated at 23°C with 16 h photoperiod for 16 weeks and rooting of bulblets were determined. In experiments, a completely randomized design with 4 factors and one replication was used. Rooting was determined as %. Data were analyzed by using ANOVA in order to test the main effects. The differences in the means were tested by Duncan's multiple comparrision test (post-hoc test) (Sokal and Rohlf, 1995). Statistical analyses were done to untransformed or angular transformed percentage data. Since shown that the result of analyses is not change in two manners, the results were examined on untransformed percentage data. Rooted bulblets were either planted out in pots containing sterilized soil mixture (sand: soil: perite in the ratio 1:2:1 by volume) or stored at 4°C for 8 weeks or maintained in MMS medium containing GA_3 (10 or 50 $mg \cdot L^{-1}$), 2% sucrose for 4 weeks before being transplanted. The development of rooted bulblets was observed during 16 weeks in a growth chamber at 23°C.

In the determination of successful transplantation, the bulblets that showed

shoot development at the end of 16 weeks and that formed green leaves were evaluated as a live; and those that became dark, dried up and did not form shoots were evaluated as dead. The rate of living explants used to was determined % transplantation success. Deferences between two independent proportions were analyses with Z-test (Sokal and Rohlf, 1995).

3. Results

The results of the effects of different levels of sucrose, AC, NAA and strength of medium on the rooting of bulblets produced *in vitro* were subjected to analysis of variance (Table 1). The most positive effect on the rooting of *G. ikariae* was obtained from medium containing 0.5 $mg \cdot L^{-1}$ NAA without being dependent on the strength of the different media used, the active charcoal additives and the sucrose concentrations. The rooting rates in media where 0.01 ve 0.1 $mg \cdot L^{-1}$ NAA was added were determined to be an average of 15.71 and 12.50% respectively (Table 2). These values were within the same group with the rooting rate obtained from the media used as a control media and not containing NAA. In media where 0.5 $mg \cdot L^{-1}$ NAA was added, there was a significant difference found in the rate of rooting compared to the control media and it produced the highest rooting value (25.42%) ($p<0.05$). For the transplantation stages experiments, rooted bulblets in 1/2 MMS medium which contained 0.5% AC, 0.5 $mg \cdot L^{-1}$ NAA and 30 $g \cdot L^{-1}$ sucrose concentration (Figure 1) and 1/8 MMS medium which contained 0.5% AC, 0.5 $mg \cdot L^{-1}$ NAA and 60 $g \cdot L^{-1}$ sucrose concentration (Figure 2) were used. Ten each bulblets rooted in these two media were used in different transplantation conditions. The results obtained at this stage are shown in Table 3. The highest transplantation success was observed on bulblets which have rooted in a medium containing 30 $g \cdot L^{-1}$ sucrose, 1/2 MMS, 0.5% AC, 0.5 $mg \cdot L^{-1}$ NAA and that were directly transferred to soil without any pre-treatment and was determined to be 28%. The rate of transplantation success was 10.2% when held at 4^oC for 8 week and 10.7% 11.7% when treated with 10 or 50 $mg \cdot L^{-1}$ GA_3 prior to the transplantation. On the other hand, at 60 $g \cdot L^{-1}$ sucrose, 1/8 MMS, 0.5% AC, 0.5 $mg \cdot L^{-1}$ NAA only those rooted bulblets which were held at 4^oC for 8 week prior to transplantation continued to survive, a 5% transplantation success. But differences between the proportions were found unsignificant statistically.

4. Discussion

The results indicated that NAA is important factor on root formation. Root formation was stimulated by increasing NAA doses. High values obtained from the combinations included 0.5 $mg \cdot L^{-1}$ NAA. Many reports have indicated the stimulatory effect of high NAA on root formation. Promotion of rooting by auxins has been reported for *Narcissus* (Hosaki and Asahira, 1980; Seabrook, 1990; Chow et al.1992).

Takayama and Misawa (1979) reported that high NAA concentrations (up to 10 $mg \cdot L^{-1}$) induced root formation in

Table 1. The results of analysis of variance of rooting rate (%) of *G. ikariae* bulblets in containing 0.01, 0.1 or 0.5 $mg \cdot L^{-1}$ of NAA, 0.2, 0.5 and 0.1% AC, 3 or 6% sucrose and 1/1, 1/2, 1/4 and1/8 MMS media.

Source	df	Sum of Squares	Means Square	F	Sig.
NAA	3	0.36291	0.12097	3.53	0.018*
AC	2	0.17063	0.08531	2.49	0.089
Medium strength	3	0.10253	0.03418	1.00	0.398
Sucrose	1	0.00094	0.00094	0.03	0.869
Error	86	2.94605	0.03426		
Total	95	3.58305			

* Difference was significant at $p<0.05$

Table 2. Descriptive statistics of the effects of the different levels of sucrose, AC, NAA and medium strength on the rooting rate of *in vitro* propagated *G. ikariae* bulblets.

Factor	Rooting rate (%)
NAA (mg·L^{-1})	
0	0.0888 ± 0.0300*
0.01	0.1571 ± 0.0429
0.1	0.1250 ± 0.0321
0.5	0.2542 ± 0.0453
AC (%)	
0.2	0.1313 ± 0.0322
0.5	0.2156 ±0.0376
1.0	0.1219 ± 0.0314
Medium Strength	
MMS	0.1130 ± 0.0315
1/2 MMS	0.1396 ± 0.0382
1/4 MMS	0.1929 ± 0,0422
1/8 MMS	0.1813 ± 0.0455
Sucrose (g·L^{-1})	
30	0.1594 ± 0.0284
60	0.1513 ± 0.0279

*: MEAN ± SEMEAN

Generally, AC is known as a root inducing inducing substance. However, there are situations in which it acts as an inhibitor. Stimulation of root growth has been severally ascribed to charcoal adsorbing some inhibitory substances or to its effect in darkening the culture medium (George and Sherington, 1984). Beside this, inhibitory effects of AC in high concentrations are mentioned that probably due to auxin absorbtion by the AC. On the other hand, in our experiments the rooting was induced by increasing of the concentratation of AC up to 0.5% and higher than 0.5% concentration of AC (1.0%) reduced the root formation of *G. İkariae*, but these effects were not statistically significant. Sucrose concentrations alone used in these experiments did not have a significant effect on rooting. Chow et al. (1992) indicated that rooting in *Narcissus* was not affected by sucrose concentration. The general impression was that the rate of rooting was positively affected with the decrease in culture medium strength. In fact, Takayama ve Misawa (1979, 1983) reported that in the rooting of *Lilium* bulblets, diluting the culture medium strength by 1/2 or 1/4 increased the growth of plants. According to our results decrease in culture medium strength increased the rooting but this increasing was not statistically significant.

The rooting medium was found to have an effect on the success of

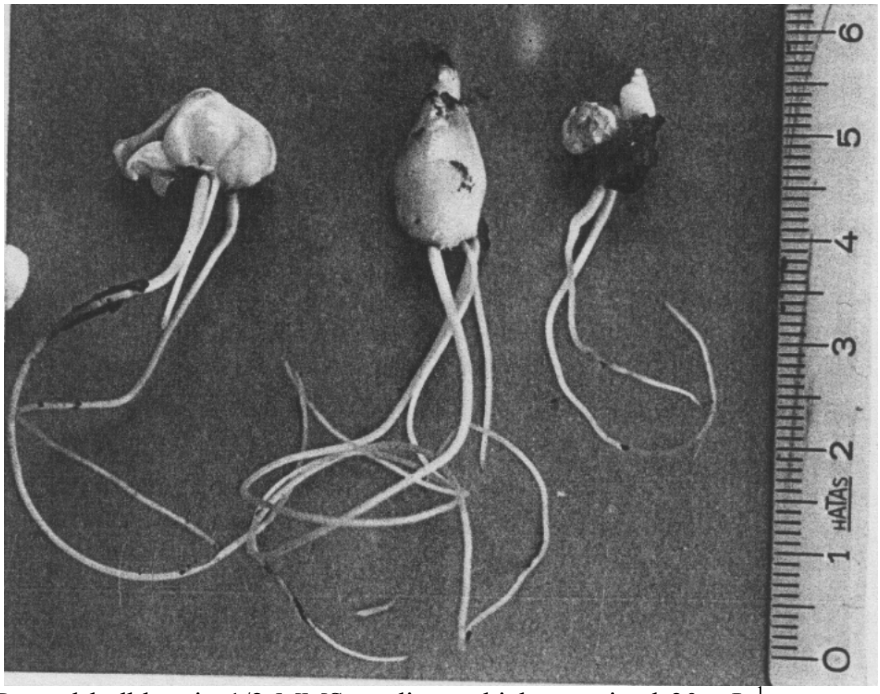

Figure 1. Rooted bulblets in 1/2 MMS medium which contained 30 g·L^{-1} sucrose, 0.5 mg·L^{-1} NAA, 0.5% AC.

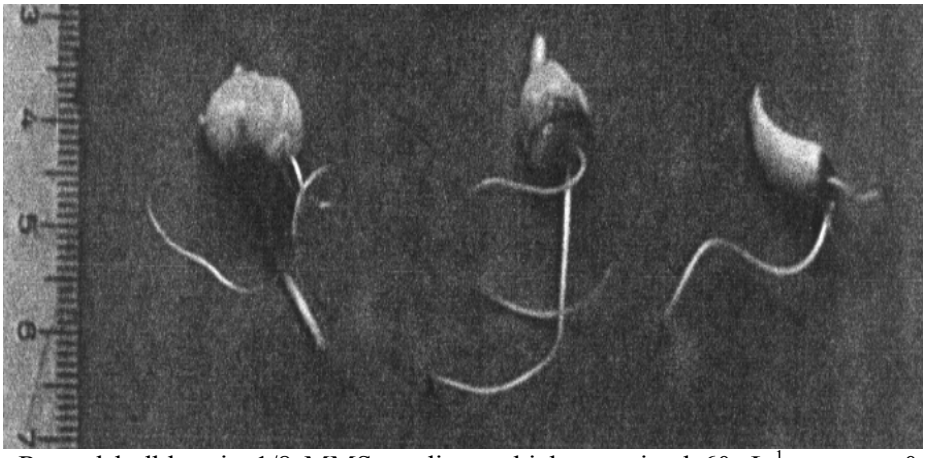

Figure 2. Rooted bulblets in 1/8 MMS medium which contained 60 ·L^{-1} sucrose, 0.5 mg·L^{-1} NAA, 0.5 %AC.

Table 3. Transplantation success (%)

	Pre-treatments			
			4 weeks GA_3 mg·L^{-1}	
Bulbil production conditions	Direct	8 weeks 4 °C	10	50
1/2MMS 30 g·L^{-1} sucrose 0.5 % AC 0.5 mg·L^{-1} NAA	28.0	10.2	10.7	11.7
1/8 MMS 60 g·L^{-1} sucrose 0.5 % AC 0.5 mg·L^{-1} NAA	0.0	5.0	0.0	0.0

Difference between two independent proportions (Z test).

transplantation of the plants. The bulblets rooted in 1/2 MMS, 30 g·L^{-1} sucrose medium attained a higher transplantation success than those rooted in a 1/8 MMS, 60 g·L^{-1} sucrose medium.

In our opinion, the sucrose dosage did not play a very great role on the transplantation success. The effect of culture medium strength was greater. In fact, in the studies of Squires et al., (1991) in which they examined the different factors affecting the transplantation success in *Narcissus* bulblets, they reported that sucrose used in the dosages of 30, 60 ve 90 g·L^{-1} were not effective on the transplantation success; however, decreasing the salts in the culture medium from full strength to half strength decreased the transplantation success to a significant extent. They also reported that two factors, low salt concentration and darkness, significantly reduced transplantation success. In our study, as the researchers mentioned, above the effect of low salt concentratation (1/8 MMS at 60 g·L^{-1} sucrose) on transplantation success was associated with reduced relative growth in culture. In addition, in our opinion, the fact that the bulblets rooted in a combination of 1/2 MMS and sucrose at 30 g·L^{-1} had a greater number and strength of roots compared with the other combination, positively affected the transplantation success. However, the rate of survival in transplantation stage was insufficient. An increased understanding of factors would seem essential to further progress in overcoming the high rate of transplantation failure for *G. ikariae.*

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