African Journal of Agricultural Research Vol. 7(17), pp. 2669-2673, 5 May, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.454
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

In vitro propagation of native Ornithogalum species in West Mediterrenean region of Turkey

Ozgul Karaguzel*, Ayse Kaya, Beyza Biner and Koksal Aydinsakir

Bati Akdeniz Agricultural Research Institute, Antalya, Turkey.

Accepted 17th April, 2012

Ornithogalum **is a popular genus especially, as an ornamental plant. In this study, eight native** ***Ornithogalum*** **species grown in West Mediterrenean Region of Turkey were cultured on MS media supplemented with combinations of BAP (1.0, 2.0, 4.0 mg L^{-1}) and NAA (0.25, 0.50 mg L^{-1}). The results indicated that the highest rate of proliferation was induced on Murashige and Skoog (MS) to which 4 mg L^{-1} BAP + 0.5 mg L^{-1} NAA (4.97 bulblets/explant) while the lowest rate of bulblet proliferation was obtained in 2 mg L^{-1} BAP + 0.25 mg L^{-1} NAA (2.27 bulblets/explant).** ***Ornithogalum umbellatum*** **showed the highest bulblet regeneration within the species. The regenerated bulblets were transferred into plastic viols containing mixture of peat moss and perlite (1:1) after 5 months.**

Key words: *Ornithogalum* spp, bulb scale explant**,** bulblet regeneration, *in vitro* propagation.

INTRODUCTION

The genus, *Ornithogalum* which belongs to family *Hyacinthaceae* (Şabudak, 1999), was widely grown in Africa, Mediterrenean, Europa and Asia naturally and contains nearly 150 species in the world (Petanidou and Vujic, 2007). Fourty four species has been represented in Turkey and 17 species of them are endemic (Davis, 1984; Ekim et al., 2000; Dusen and Deniz, 2005; Uysal et al., 2005). *Ornithogalum* species is an impressive ornamental plant due to its attractive white flowers and used as medicinal plants (Asimgil, 2003). Some *Ornithogalum* species (*Ornithogalum comosum* L., *Ornithogalum lanceolatum* Labill., *Ornithogalum latifolium* Baker, *Ornithogalum pyrenaicum* L., *O. narborense L., Ornithogalum oligophyllum* Clarke and *Ornithogalum sibthorpii* W.Greuter) have potentially an economic importance since it is consumed as vegetable in Turkey (Baytop, 1997), whereas, it has not sufficently been considered to propagation.

Ornithogalum species are vegetatively propagated using mother bulbs, but the rate of propagation of these bulbs is very slow. Approximetly 4 to 6 bulblets are annualy produced from a mother bulb (Naik and Nayak, 2005). Therefore *in vitro* techniques were used for rapid propagation of bulbs. Several studies have been carried out *in vitro* micro-propagation protocols of some culture *Ornithogalum* species (Hussey, 1976; Nel, 1981; Yanagawa and Ito, 1988; Rensburg et al., 1989; Landby and Neiderwieser, 1992; Ziv and Lilien-Kipnes, 2000; Karuiki and Kako, 2003; Malabadi and van Staden, 2004; Naik and Nayak, 2005; Suh et al., 2005). At the same time, micro-propagation of some native *Ornithogalum* species such as *O. umbellatum* L. (Nayak and Sen, 1995)*, O. ulouphyllum* Hand-Mazz (Ozel et al., 2008), *O. plathyllum* Boiss (Ipek et al., 2006)*, O. oligophyllum* E. D.Clarke (Ozel and Khawar, 2007) has been reported. However, there are no reports *in vitro* for the propagation a large number of the native *Ornithogalum* species up to now. In the present study, a new propagation protocol for bulblet regeneration from bulb scale explants of eight native *Ornithogalum* species in the West Mediterranean

*Corresponding author. E-mail: tezkara@yahoo.com.

Abbreviations: BA, Benzyl adenin; **BAP,** benzyl amino purin; **NAA,** naphthalene acetic acid; **NaOCl,** sodium hypochlorite; **KOH,** potassium hydroxide; **HCl,** hydrochloric acid.

Region of Turkey was investigated.

MATERIALS AND METHODS

Plant material and explant source

The *Ornithogalum* species (O. *umbellatum* L.*, O. oligophyllum* E. C. Clarke*, O. sigmoideum* Freyn. Sint.*, O. narborense* L.*, O. pyrenaicum* L.*, O. lanceolatum* Labill.*, O. isauricum* O. D. Düşen and H. Sümbül *'endemic'* and *O. nutans* L.) were collected between April and June, 2006 in West Mediterranean Region of Turkey.

Ornithogalum bulbs were washed under running tap water for half an hour to remove the mud and dirt. Firstly, the bulbs were sterilized by treatment for 10 min in a 10% commercial bleach (5 to 6% NaOCl) + 172 ml Tween 20 per 100 ml. Then for 30 min in 80% commercial bleach (5 to 6% NaOCl) + 172 ml Tween 20 per 100 ml commercial bleach with continuous stirring using magnetic stirrer. Finally, they were rinsed in sterile distil water thrice. After removing the outer scales of the bulbs, explants were longitudianally cut under sterile conditions to obtain about 1 to 2 cm four scale segments including the basal plate.

Medium and culture conditions

The explants were cultured on MS medium (Murashige and Skoog, 1962), supplemented with 1.0, 2.0, 4.0 mg L^{-1} BAP and 0.25, 0.50 mg L^{-1} NAA. The pH values of all media was adjusted to 5.7 with 1 N KOH or 1 N HCl before adding 30 g L^{-1} sucrose and 7 g L^{-1} agar (Sigma, 1296) and autoclaved at 1.2 atm and at 121°C for 20 min. Petri dishes were wrapped with strech film and then placed at 25 ± 1°C by 16 h photoperiod under 100 µmol m^{-2} s^{-1} light intensity supplied with white fluorescent light. The bulblets were subcultured every month onto fresh medium for aproximately three months and later transferred to the MS medium containing no plant growth regulators for induction of rooting. Each treatment consisted of 5 explants and the experiments were repeated thrice.

Data collection and statistical analysis

The number of bulbs per explant and survival rate of bulblet transfered to soil were determined in eight different *Ornithogalum* species at the end of the experiment. All data were statistically analyzed according to a completely randomized design (Gomez and Gomez, 1984) and variance analysis (ANOVA) was done. Means were calculated and Duncan's multiple range test at a significance level of 5% was compared.

RESULTS

Explants cultured on MS medium supplemented with different combinations of plant growth regulators displayed proliferation within 15 to 20 days, by small bulblets which were developed on the scales. Bulblets were produced directly on the bulb scales or the basal plates (Figure 1). The results indicated that the effect of plant growth regulators on the number of bulblet regeneration for each species were statistically different ($P<0.01$). The mean number of bulblets per explant on *Ornithogalum* species cultured on MS medium containing different concentrations of BAP and NAA are presented in Table 1. The highest numbers of bulblets 4.97 per explant were recorded on MS media containing 4.0 mg L^{-1} BAP + 0.50 mg L^{-1} NAA and 4.67 bulblets per explant on 1 mg L^{-1} BAP + 0.25 mg L^{-1} NAA. The least number of 2.27 bulblets per explant was recorded by supplementation with 2.0 mg L^{-1} BAP + 0.25 mg L^{-1} NAA in culture medium. The maximum number of bulblets was 21.67 on MS medium containing 4.0 mg L^{-1} BAP + 0.50 mg L^{-1} in *O. umbellatum* (Table 1 and Figure 1a). Limited number of bulblet (respectively, 0.11 and 0.89) was obtained from *O. nutans* and *O. isauricum* (endemic) species due to the intensive infection on mediums. Formed bulblets were washed under running tap water and were transfered into plastic viols containing mixtures of peat moss and perlite (1:1) at the end of 5 months and maintained by watering one time for two days in greenhouse (Figure 2). The survival ratios of bulblets (4 to 5 mm) varied between 25 and 100% (Table 1).

DISCUSSION

Ornithogalum species are generally propagated from bulbs. Seed propagation is not practical because it needs at least 4 to 5 years growth period from seed to the flowering stage. However, the rate of vegetative propagation of bulbs is very slow, rendering the possibility for growing of 1 to 2 plants in a year. For this reason, *in vitro* propagation is an alternative method for the multiplication of bulbous plants including *Ornithogalum* species.

In previous studies, different plant parts and organs such as bulb scale, thin layer cells, matured seeds, matured leaves, shoot tips, perianth, stem nodes and immature embryos were used as an explant material for *in vitro* propagation of *Ornithogalum* species (Hussey, 1976; Nayak and Sen, 1995; Malabadi and van Staden, 2004; Ozel and Khawar, 2007). Bulb scale explant having the highest growth potential to produce new bulblets were previously used as explant sources (Nayak and Sen, 1995; Zaidi et al., 2000; Ozel et al., 2008). Bulb scale explants of bulbous plants have 2 to 4 leaves for *in vitro* production (Mirici et al., 2005; Nasircilar et al., 2011; Karaoğlu, 2010). Different mediums were used for *in vitro* propagation of bulbous plants. MS (Murashige-Skog, 1962) medium is preferred for bulb propagation. However, N_6 (Chu et al., 1975) medium was used for immatured embryo culture. Plant growth regulators are also important user agent to stimulate bulblet regeneration from bulb scale in rapid propagation. NAA as an auxin, BA and BAP as a cytokinin source were used in propagation from bulb scale explants of various *Ornithogalum* species and different results were published (Nel, 1981; Yanagawa and Ito, 1988; Suh et al., 2005; Naik and Nayak, 2005; Ozel et al., 2008).

The highest bulblet regeneration of *O. ulophyllum* was

Figure 1. Bulblet regeneration from 4 scale explants eight different *Ornithogalum* species "a) 4.0 mg L^{-1}BAP+0.50 mg L^{-1} NAA *O. umbellatum,* b) 1.0 mg L^{-1} BAP+ 0.25 mg L^{-1} NAA *O. oligophyllum,* c) 1.0 mg L^{-1} BAP+ 0.25 mg L^{-1} NAA *O. sigmoideum,* d) 4.0 mg L^{-1} BAP+0.25 mg L^{-1} NAA *O. narborense*, e) 0 mg L^{-1} BAP+ 0.50 mg L^{-1} NAA *O. lanceolatum*, f) 1.0 mg L^{-1} BAP+ 0.50 mg L^{-1} NAA *O. isauricum,* g) 1.0 mg L^{-1} BAP+ 0.50 mg L^{-1} NAA *O. nutans,* h) 1.0 mg L^{-1} l BAP+ 0.25 mg L^{-1} NAA *O. pyrenaicum".*

Table 1. Effects of growth regulators on bulblet regeneration from 4 scales and mean number of bulblets per explant on *Ornithogalum* species.

Species	Mean number of bulblets per explant						Mean of species	Survival rate of bulblet transfered to soil (%)
	Plant growth regulators (mg L^{-1})							
	1 BAP + 0.25 NAA	1 BAP+0.5 NAA	2 BAP + 0.25 NAA	2 BAP + 0.5 NAA	4 BAP + 0.25 NAA	4 BAP + 0.5 NAA		
O. umbellatum	19.00^{Aax}	7.00^{Ba}	3.67^{Ba}	6.67^{Ba}	6.67^{Ba}	21.67^{Aa}	10.78^{**}	51
O.oligophyllum	14.33^{Ab}	5.33^{Bac}	4.00^{Ba}	5.00^{Bab}	2.00^{Bbc}	4.33^{Bbc}	5.83	29
O.sigmoideum	3.00^{Ac}	2.33^{Abd}	2.67^{Aa}	1.67^{Abc}	2.33^{Ab}	4.33^{Ab}	2.72	58
O.narborense	3.67^{ABc}	6.33^{Aab}	4.00^{Aba}	4.33^{ABac}	1.67^{Bb}	6.33^{Ab}	4.39	67
O.pyrenaicum	1.33^{Ac}	1.67^{Acd}	2.67^{Aa}	1.67^{Abc}	2.67^{Abc}	2.33^{Abc}	2.06	41
O.lanceolatum	3.00^{Bc}	7.33^{Aa}	2.33^{Ba}	2.67^{Bac}	2.67^{Bb}	5.67^{ABb}	3.94	28
O.isauricum (*endemic*)	0.00^{Ac}	1.33^{Acd}	0.00^{Aa}	1.33^{Abc}	2.67^{Ac}	0.00^{Ac}	0.89	20
O.nutans	0.00^{Ac}	0.00^{Ad}	$0.00A^{a}$	0.00^{Ac}	0.00^{Ac}	0.67^{Ac}	0.11	100
Mean of plant growth regulators	4.67^{**}	3.97	2.27	2.77	2.30	4.97	3.49	

Mean separation within columns by Duncan's multiple range test, at 0.05 level; ^{ns} No statistical difference at $P < 0.05$ and $P < 0.01$, * Statistical difference at $P < 0.05$, ** Statistical difference at $P < 0.01$, ^{X}: capitals show the comparison between the averages given horizontally (along the line) and the small characters show the comparison between the averages given vertically (along the column).

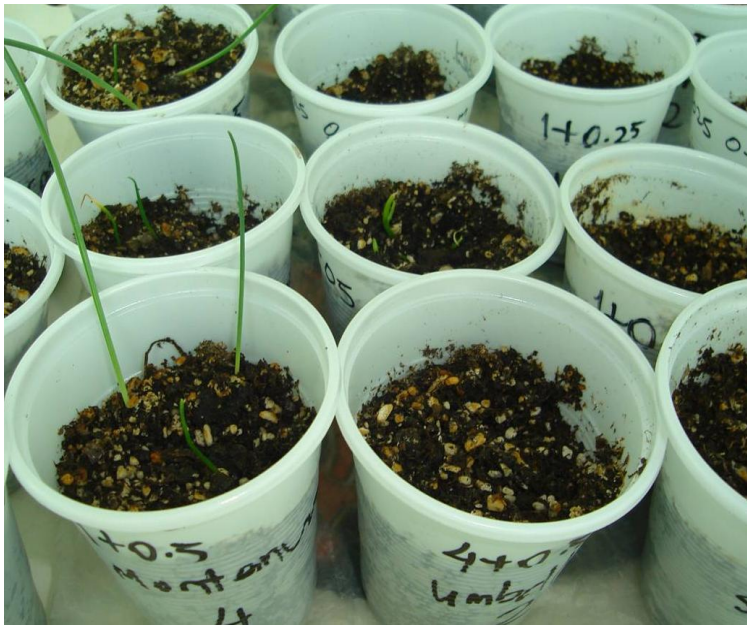

Figure 2. Plants growth from bulblets in growing media containing peat moss and perlite (1:1).

obtained with a medium containing 2.0 mg L^{-1} BAP + 0.50 mg L^{-1} NAA. Number of bulblet per plant was 4.83. (Ozel et al., 2008). In our study, number of bulblet per plant in *O.umbelatum* was 21.67 while, 2.06 to 5.83 values were recorded in the other excluded species that encountered infection. The most constructive results were also obtained from MS medium with 2.0 mg L^{-1} BA + 1.0 mg L^{-1} NAA (10.4 bulblets/explant) in *O. virens* as reported by Naik and Nayak (2005). Correspondingly, a remarkable increase in the regeneration of cultured *O. arabicum* bulblets by 5.0 mg L^{-1} BA + 0.01 mg L^{-1} NAA on solid White's medium was detected (Yanagawa and Ito, 1988). In other studies, development of the highest number of shoots from bulb scale explants on *Ornithogalum* hybrid cultured on MS medium with 1.5 mg L^{-1} BA + 0.50 mg L^{-1} NAA was also reported (Suh et al., 2005). In our present study, the highest bulblet formation was achieved by 4.0 mg L^{-1} BAP + 0.50 mg L^{-1} NAA at eight different *Ornithogalum* species. These obtained incoherent findings can be associated with differences of the genotypes, explants and concentrations of growth regulators used in mediums. During the studies, bacterial and fungal contaminations were observed on *O. nutans* and *O. isauricum* culture medium and limited number of bulblets were obtained from these species. The presence of heavy bacterial and fungal contamination risks were reported if the bulbs will be used as a source of explant (Langens-Gerrits et al., 1998; Ziv and Lilien-Kipnis, 2000; Mirici et al., 2005). Also, Karaoğlu (2010) stated that despite all surface sterilizations done in some bulbous plants, diseases were not prevented and they resulted from endogenic plants.

In this study, it was found that the number of bulblets were significiantly increased with the addition BA and NAA into medium culture. In conclusion, from the ongoing results, it may be concluded that a simple and rapid protocol can be established for propagation of eight *Ornithogalum* species which were grown in West Mediterranean Region of Turkey.

ACKNOWLEDGEMENTS

This research was supported by the Scientific and Technical Research Council of Turkey (TUBITAK; Project no TOVAG 104O327). The authors wish to thank Prof. Dr. İbrahim BAKTIR, Prof. Dr. Osman Karagüzel and Associate Prof. Dr. Ö. Baysal.

REFERENCES

Asimgil A (2003). Şifali Bitkiler. Hayat-Sağlik 352 s, (in Turkish).

Baytop T (1997). Türkçe Bitki Adlari Sözlüğü, Atatürk Kültür, Dil ve Tarih Yüksek Kurumu, Türk Dil Kurumu Yayinlari, 578, Ankara, (in Turkish).

Chu CC, Wang CC, Sun CS (1975). Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci. Sin., 18: 659-668.

Davis PH (1984). Flora of Turkey and The East Agean Islands, University Pres Edinburgh, Vol.:8.

Düşen O, Deniz İG (2005). *Ornithogalum sumbulianum* (*Hyacinthaceae*), a new endemic species from South West Anatolia. Pak .J. Bot., 36(4): 33-36.

Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adigüzel N (2000). Türkiye Bitkileri Kirmizi Kitabi. (Eğrelti ve tohumlu Bitkiler), Ankara,196 s, (in Turkish).

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research. In: An Int. Rice Res. Inst. Book. John Wiley and Sons Inc., New York, p. 680.

Hussey G (1976) Plantlet regeneration from callus and parent tissue in *Ornithogalum* thyrosoides. J. of Exp. Bot., 27(97): 375-380.

Karaoğlu C (2010). Soğanli Bitkiler ve *In vitro* Hizli Çoğaltim. Tarla Bitkileri Merkez Araştirma Enstitüsü Dergisi 19(1-2):24-29.

Kariuki W, Kako S (2003). Micropropagation of *Ornithogalum saundersiae* Bak. Acta Hort., 624: 521-526.

Landby PA, Neiderwieser JG (1992). *In vitro* propagation of *Ornithogalum* 'Rollow'. Afr. Soc. Hort. Sci., 2(1):50-54.

Langens-Gerrits M, Albers M, Klerk GJ (1998). Hot water treatment before tissue culture reduces initial contamination in *Lilium* and *Acer.* Plant Cell Tiss. Org. Cult., 52: 75-77.

Malabadi RB, Van Staden J (2004). Regeneration of *Ornithogalum in vitro.* Afr. J. Bot., 70(4): 618-621.

Murashige T., F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473–497.

Mirici S, Parmaksiz İ, Özcan S, Sancak C, Uranbey S, Sarihan E.O, Gümüşçü A, Gürbüz B, Arslan N (2005). Efficient *in vitro* bulblet regeneration from immature embryos of endangered *Stenbergia fischeriana.* Plant Cell Tiss. Org. Cult., 80: 239-246.

Nasircilar, A, Mirici, S, Karagüzel, Ö, Eren, Ö, Baktir, İ (2011). *In vitro* propagation of endemic and endangered *Muscari mirum* from different explant types. Turk. J. Bot., 35: 37-43.

Nel DD (1981). Rapid propagation of Ornithogalum hybrid *in vitro.* Agroplantae, 13(3): 83-84.

Nayak S, Sen S (1995). *In vitro* propagation of *Ornithogalum umbellatum* through direct organogenesis. Ind. J. Exp. Bio., 33(2): 144-146.

Naik PK, Nayak S (2005). Different modes of plant regeneration and factors affecting *in vitro* bulblet production in *Ornithogalum virens.* Sci. Asia, 31: 409-414.

Ozel ÇA, Khawar KM (2007). *In vitro* bulblet regeneration of *Ornithogalum oligophyllum* E.D. Clarke Using twing scale bulb explants. propagation of ornamental plants. Propag. Ornam. Plants, 7(2): 82-88.

Ozel ÇA, Khawar KM, Karaman S, Ateş MA, Arslan O (2008). Efficient *in vitro* multiplication in *Ornithogalum ulouphyllum* Hand.-Mazz. from twin scale explants. Sci. Hort., 116: 109-112.

Petanidou T, Vujic A (2007). Genetic diversity & mutual dependence *of Ornithogalum* plants and *Merodon* hoverflies across a climatic gradient within the Mediterranean.http://www.alarmproject.net net.ufz.de/documents/fsn_protocol_2007/2007_03.pdf.

Rensburg JGJ, Vcelar BM, Landby PA (1989). Micropropagation of *Ornithogalum maculatum*. South Afr. J. Bot., 55(1): 137-139.

Suh KJ, Lee W, Lee A (2005). New plantlet proliferation and bulbing promotion *in vitro* of *Ornithogalum* hybrid. Acta Hort., 683: 155-163.

Şabudak T (1999). Trakya bölgesinde yetişen *Ornithogalum umbellatum* (*Hyacinthaceae*) L. bitkisinin kimyasal bakimdan incelenmesi. Trakya Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. p.104, (in Turkish).

Uysal T, Ertuğrul K, Dural H (2005). A new species of *Ornithogalum* (Liliaceae) from South Anatolia, Turkey. Bot. J. Linnean Soc., 148: 501–504.

Yanagawa T, Ito I (1988). Differences in the capacity for bulblet regeneration between bulb scale explants excised from different parts of Ornithogalum bulbs. J. Jap. Soc. Hort. Sci., 57(3): 454-461.

Zaidi N, Khan NH, Zafar F, Zafar SI (2000). Bulbous and cormous monocotyledonus ornamental plants *in vitro*. Quat. Sci. Vis., 6(1): 58-73.

Ziv M, Lilien-Kipnes H (2000). Bud regeneration from inflorescense explants for rapid propagation of geophytes *in vitro.* Plant Cell Rep., 19(9): 845-850.