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AN ALTERNATIVE PLANT PROPAGATION AND CONSERVATION PROCESS FOR *IRIS PAMPYHLICA* AN ENDEMIC AND ENDANGERED GEOPHYTE

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Abstract

Flowering bulbs have been used for ornamental purposes throughout history. Nearly 1000 geophytes are naturally grown in Turkey and many of them harvested from their natural habitats. Native flower bulbs are exported and this export has increased from year by year. Due to uncontrolled and excessive collection from wild, a large number of them are threatened with complete extinction. For this reason, different cultural propagation methods must be developed to assist the conservation of these plants. The genus *Iris* contains essential oils, has attractive flowers, and can be used ornamental and medicinal purposes. Vegetative or generative production is not efficient enough to reproduce *Irises,* however *in vitro* micropropagation is an alternative way to protect and propagate them. In this study, tissue culture techniques were applied for the vegetative propagation of *Iris pamphylica* which is an endemic and endangered geophyte of Turkey. Fresh bulbs and immature embryos were cultured on Murashige-Skoog (MS) medium supplemented with various combinations of BAP (6-benzylaminopurine), NAA (α naphtalenacetic acid), 2,4-D (2,4-dichlorophenoxy acetic acid) or picloram (4-amino-3,5,6-trichloropicolinic acid). While plant production was achieved from bulb explants via direct organogenesis, the only callus formation or plant regeneration through indirect organogenesis was obtainable from immature embryo explants of *I. pamphylica.* The best results for shoot formation were acquired in an MS medium containing 2 mg L^{-1} BAP and 0.25 mg L^{-1} NAA for bulb explants. BAP- NAA combinations in the culture medium are more effective than 2,4-D or picloram in immature embryo culture. It was noted that immature embryo explants were more efficient than bulb explants for *in vitro* plant regeneration of *I. pamphylica.*

Key Words: *Iris pamphylica, alternative propagation, bulb, immature embryo*

Introduction

The genus *Iris* which is a member of *Iridaceae* family includes over 300 species, many of which have importance in horticultural and pharmaceutical industry (Kerasa et al., 2009; Wang et al., 1999a; Jevremović and Radojević, 2002). Several species of the genus contains volatile oils and xanthones that can be used for making perfume and medicine (Wang et. al., 1999a; Jevremović and Radojević, 2002; Al-Gabbiesh et. al., 2006). Various *Iris* species, having attractive flowers with different shades and colours, are also used for ornamental purposes in gardening and landscaping (Francescangeli, 2009; Jevremovći and Radojević, 2002) The propagation of *Iris* species are generally performed vegetatively by splitting rhizomes or bulbs (Jéhan et. al, 1994). Large scale production of *Irises* limited due to issues such as poor seed production, germination problems, cross pollinations and the long juvenile period in the plant's development. For these reasons, vegetative production or reproduction by seeds alone, is not appropriate for commercial production of the desired *Iris* species (Boltenkov et.al., 2007; Wang et al., 1999a). *In vitro* micropropagation which is used for production of most herbaceous plants is also an alternative technique in *Iris* production, and

is, compared with conventional breeding, a more efficient and rapid method (Wang et al. 1999b; Al-Gabbiesh et. al., 2006; Boltenkov et. al., 2007). Several *Iris* species such as *Iris hollandica* (Hussey, 1976), *I. pumila* (Radojević et. al., 1987), *Iris ensata* (Yabuya et. al., 1991; Boltenkov and Zarembo, 2005), *I. setosa* (Radojević and Subotić, 1992; Boltenkov and Zarembo 2005), *Iris stenophylla* (Nasircilar et.al 2011) and *I. sanguinea* (Boltenkov and Zarembo 2005) have been propagated from flower stem (Hussey, 1976) mature embryos (Radojević et.al., 1987), scapes (Yabuya et.al., 1991), different sections of flowers (Boltenkov and Zarembo, 2005) and immature embryo explants (Nasircilar et. al, 2011) via *in vitro* plant regeneration. *Iris pamphylica* (Figure 1a) which is a locally endemic and endangered geophyte of Turkey, displays beautiful purple flowers (Figure 1b). This species which has a restricted distribution between the Manavgat-Akseki districts of Antalya (Mathew, 1985; Kandemir et. al., 2011), and is listed in the endangered category of the Red Data Book of Turkish Plant (Ekim et. al., 2001). Due to its ornamental value and it is danger of extinction, strict protection and efficient production method should be developed. Although various *Iris* species were propagated via tissue culture, *in vitro* or *ex vitro* culture of *I. pamphylica* has not been to date. In this study an alternative production method, crucial for the protection of this endangered species is outlined.

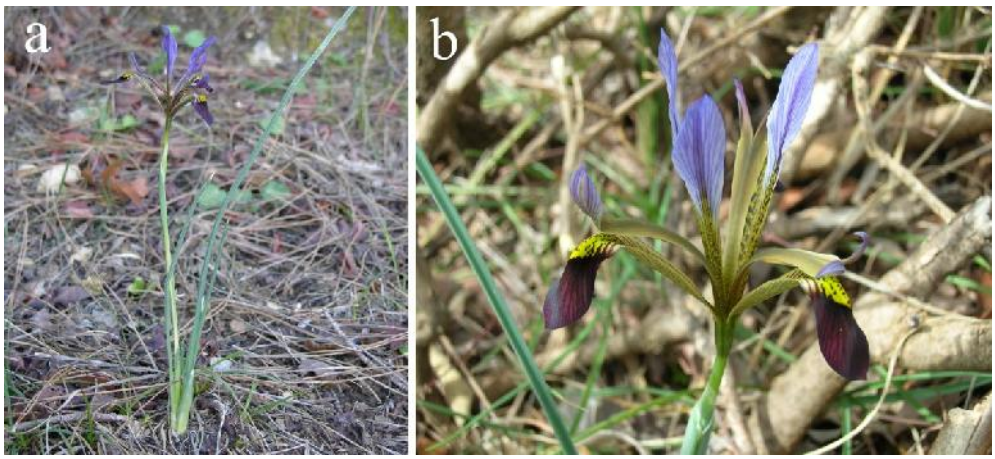

Figure 1. a) *Iris pamphylica* plant in their natural habitat b) The flower of *Iris pamphylica*

Material and Methods

Plant materials

Iris pamphylica plants were collected from two different locations between the Manavgat-Akseki districts of Antalya in early spring. Two different parts; fresh bulbs and immature embryos of *I. pamphylica* were used as the explant sources for *in vitro* regeneration of the plant.

Sterilization and preparation of the bulb explants

After the bulbs of *I. pamphylica* were seperated from the plant, the roots and the outher scales were peeled and discarded. Before surface sterilization, the bulbs were washed in detergent thoroughly under running tap water. Surface sterilization of the bulbs was done in 80% commercial bleach with continuous stirring for 30 minutes and rinsed 3 times in sterile distilled water. After sterilization, bulbs containing the basal disc were cut into two or four sections depending on the size of the bulb. The explants were aseptically inoculated onto the Murashige and Skoog' s basal medium (Murashige and Skoog, 1962) supplemented with 1, 2 or 4 mg L^{-1} 6-benzylaminopurine (BAP) and 0.25 mg L^{-1} α-napthalenacetic acid (NAA), 30 g L^{-1} sucrose and 7g L^{-} agar. The pH of all media was adjusted to 5.7 before autoclaving at

121ºC for 20 min. The cultures were kept at 25^0C±1 and under a 16-h (day)/8-h (night) photoperiod. Explants were transferred to fresh medium every month.

Sterilization and preparation of the immature embryo explants

Fruits containing immature zygotic embryos were immersed in 80% commercial bleach for 20 minutes and rinsed 3 times in sterile distilled water for surface sterilization. Immature zygotic embryos were removed aseptically from immature seeds using forceps under dissection microscope. The explants were placed on MS basal medium containing 1, 2 or 4 mg L^{-1} 6-benzylaminopurine (BAP) and 0.25 or 0.5 mg L^{-1} α-napthalenacetic acid (NAA), 1.0, 2.0 mg L^{-1} 2,4-D or 1.0, 2.0 mg L^{-1} picloram, 30 g L^{-1} sucrose and 7 g L^{-1} agar. The pH of all media was adjusted to 5.7 before autoclaving at 121ºC for 20 min. The cultures were kept at 25^0C ±1 and under a 16-h (day)/8-h (night) photoperiod. Explants were subcultured every month. All the treatments were replicated three times with 3 explants per replication. Callus and shoot formation rates from both explant types are expressed as a percentage.

Results and Discussion

Some plant species are faced with complete extinction due to destruction of their natural habitats with various reasons and excessive amount of illegal collection from the nature (Kesici et.al., 2010; Karagüzel et.al., 2012). *I. pamphylica*, an endemic *Iris* species, is endangered due to the same reasons. It has a restricted distribution area and propagates limited numbers of seeds. During the three years of this study, only a small number of seed bearing plants have been found in the field trips. *In vitro* cultivation techniques were developed as an alternative system to protect rare and endangered species, including *Irises.* In previous studies, *in vitro* plant regeneration via embryogenesis or organogenesis after callus induction had been reported in various *Iris* species (Hussey,1976; Wang et. al., 1999a; Al-Gabbiesh et.al., 2006; Boltenkov et. al., 2007; Kerasa,et.al., 2009;). In this study two different parts, fresh bulbs and immature embryo of *I. pamphylica* were used the explants sources.

Shoot Regeneration from Bulb Explants

Due to an endangered species, only a few bulbs of *I. pamphylica* were collected in their natural habitats and a limited number of examinations were performed. The explants which were prepared by cutting two or four pieces with basal disc were cultured on MS medium supplemented with various concentrations of BAP (6-benzylaminopurine) and NAA (α naphtalenacetic acid). Shoot regeneration was formed on the basal disc of the bulb explants and plant production was achieved via direct organogenesis. The percentage of shoot regeneration varied according to the medium which were supplemented with different amount of BAP and NAA (Table 1). The highest shoot formations were obtained in the MS medium containing 2 mg L^{-1} BAP and 0.25 mg L^{-1} NAA. Shoot formation frequency was 50 % in this medium.

Table 1. The percentage of shoot formation from bulb explants of *I pamphylica*

Plant growth regulators (mg L^{-1})		Frequency (%) of shoot formation
BAP	**NAA**	
1	0 .5	33.33
2	0.5	33.33
4	0.5	16.66
1	0.25	0.00
2	0.25	50.00
4	0.25	16.66

Van der Linde et al. (1988) obtained *in vitro* shoot formation from bulb scale explants of *Iris hollandica* on a half concentrations of MS salts at 20°C in the dark. Nasircilar et.al (2011) also achieved *in vitro* plant regeneration from bulb explants of *Iris stenophylla* on MS medium supplemented with 1,2,4 mg L^{-1}BAP and 0.25 mg L^{-1} NAA. 1 mg L^{-1} BAP and 0.25 mg L^{-1} NAA was found the best hormonal composition for shoot regeneration in their study. Although bulb explants are commonly used as an explants sources to produce many geophytes, bacterial and fungal contaminations were the main problem in *in vitro* plant propagation from the bulbs (Mirici et. al., 2005). Because the same problem, no shoot regeneration was obtained on MS medium containing 1 mg L^{-1} BAP and 0.25 mg L^{-1} NAA from the bulb explants of *I. pamphylica*.

Callus and Shoot Regeneration from Immature Embryo Explants

The *in vitro* tissue culture system provides an artificial microenvironment for different explant types to develop and grow. Factors such as media composition, light, humidity, temperature and explant type affect to callus and plant regeneration *in vitro* conditions (Al-Gabbiesh et.al., 2006). Culture mediums of auxin and cytokinin combinations are especially effective in callus formation and plant regeneration (Wang et.al., 1999a; Al-Gabbiesh et.al., 2006) In our study; BAP, NAA, picloram and 2,4-D were used as the plant growth regulators for callus and shoot formation from immature embryo explants. Although callus formation (Figure 2) was achieved in all tested media, shoot regeneration through indirect organogenesis (Figure 3b) were obtained only on MS medium supplemented with BAP and NAA (Table 2). BAP and NAA combinations in the culture medium resulted in higher callus and shoot formation frequency in comparison with picloram or 2,4-D (Table 2). The colour, structure and growth characteristics of the callus may differ according to the plant species (Al-Gabbiesh et. al., 2006). In our study, after two months of culture initiation, yellow (Figure 2), creamy or white (Figure 3a) rigid callus structures were obtained in different hormonal combinations. Previous studies reported that the presence of 2,4-D and kinetin in the culture medium stimulated callus formation in some iris species (Boltenkov et.al., 2007; Shimizu et al.1997; Wang et.al., 1999a). After callus formation, shoots were developed on callus surface and plant regeneration was acquired via indirect organogenesis (Figure 3b). No shoots were obtained in the presence of 2,4-D or picloram. Boltenkov et. al. (2007) also reported that adventitious shoot formation from the callus of *I.ensata* required the absence of 2,4-D in the medium similarly, thus cytokinins are more important than auxins in the regeneration of this species.

Table 2. Callus and shoot formation from immature embryo explants of *I pamphylica*

Plant growth regulators (mg L^{-1})		Frequency (%) of callus formation	Frequency (%) of shoot formation
BAP	**NAA**		
1	0.5	66.66	33.33
2	0.5	100.00	100.00
4	0.5	100.00	100.00
1	0.25	100.00	100.00
2	0.25	100.00	100.00
4	0.25	100.00	100.00
Picloram			
1		22.22	0.00
2		44.44	0.00
2,4-D			
1		66.66	0.00
2		66.66	0.00

Figure 2. Callus formation from immature embryo explants of *Iris pamphylica* on MS medium containing 2 mg/l picloram

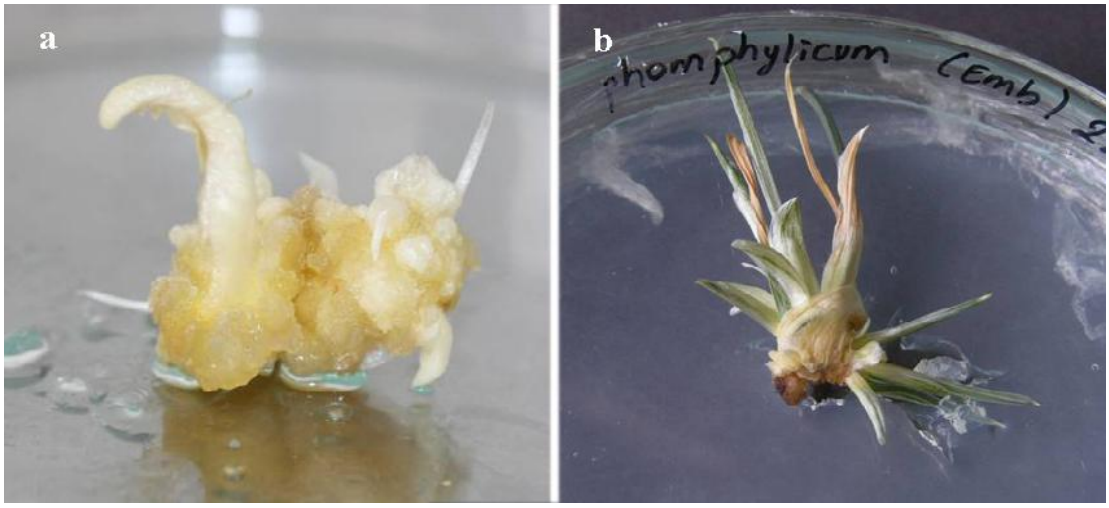

Figure 3.a) Callus and b) shoot formation from immature embryo explants of *Iris pamphylica* on MS medium containing 2 mg L^{-1} BAP and 0.25 mg L^{-1} NAA

Conclusion

The objective of this study was described as an alternative plant production system for conservation of *Iris pamphylica* via *in vitro* micropropagation. The results showed that the immature embryo explants were more efficient than bulb explants for in vitro plant regeneration of *I. pamphylica.*

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