ACTA BIOLOGICA CRACOVIENSIA Series Botanica 56/1: 97–103, 2014
DOI: 10.2478/abcsb-2014-0010

PLANT REGENERATION VIA ORGANOGENESIS AND SOMATIC EMBRYOGENESIS IN *VERBASCUM SINUATUM* L.

ROYA KARAMIAN* AND FATEMEH GHASEMLOU

Department of Biology, Faculty of Science, Bu-Ali Sina University, P. O. Box 65175/4161, Hamedan, Iran

Received: July 6, 2013; revision accepted January 3, 2014

The genus *Verbascum* L. belongs to the family Scrophulariaceae and its members are used as medicinal herbs in traditional medicines worldwide. In this study we achieved plant regeneration in *Verbascum sinuatum* L. via organogenesis and somatic embryogenesis by culture of mature embryos. Embryogenic and nonembryogenic calli were induced from mature embryos on Murashige and Skoog (MS) medium supplemented with different concentrations of benzyl adenine (BA) and α-naphthalene acetic acid (NAA) (but not for 1.5 and 3 mg l^{-1} NAA). For multiplication of somatic embryoids and differentiation of shoot buds, yellow and friable embryonic calli were transferred to MS medium containing 30 g/l sucrose, 0.5 mg l^{-1} charcoal and 0.1 or 1 mg l^{-1} 2,4-dichlorophenoxy acetic acid (2,4-D) or to MS medium containing 60 g l^{-1} sucrose, 50 mg l^{-1} casein hydrolysate (CH), 0.5 mg l^{-1} kinetin (Kin), 5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} charcoal. Shoot multiplication and plantlet regeneration were achieved by transferring shoot buds to MS medium supplemented with 1 mg l^{-1} BA or Kin.

Key words: Caulogenesis, embryogenic callus, mature embryos, micropropagation, rhizogenesis, *Verbascum*.

INTRODUCTION

The genus *Verbascum* L. belongs to the tribe Verbasceae (Valdes, 1987) of the family Scrophulariaceae and has its main centers of diversity in Turkey, Iran and Pakistan (Zohary, 1973; Huber-Morath, 1978; Valdes, 1987). With about 360 species worldwide (Judd et al., 2002) and 42 species in Iran (Sharifnia, 2007), it is the largest genus within the Scrophulariaceae. As hybridization is a very frequent phenomenon within *Verbascum* (Huber-Morath, 1978; Karaveliogullari et al., 2004), high morphological variation complicates delimitation of species. *Verbascum* species are adapted to the various habitats in different regions, including rocky mountains, open forests, roadsides and river banks. The genus *Verbascum* is known for the presence of a variety of compounds. Among those that have been isolated are saponins (Hartleb and Seifert, 1994; Tatli et al., 2004; Karamian and Ghasemlou, 2014), alkaloids (Dranarov and Hais, 1997), flavonoids (Afifi et al., 1993; Karamian and Ghasemlou, 2013a), iridoid glycosides (Tatli et al., 2003; Tatli and Akdemir, 2004) and phenylethanoids (Akdemir et al., 2004).

A wide range of medicinal properties are attributed to *Verbascum* species (Vijayavitthal et al., 1998; Tuzlaci and Erol, 1999; Konoshima et al., 2000; Sezik et al., 2001; Stevenson et al., 2002; Turker and Camper, 2002; Ahmed et al., 2003; Senatore et al., 2007; Amirnia et al., 2011; Karamian and Ghasemlou, 2013b).

Plant tissue culture is one of the most important conservation techniques for production of plant material required for different purposes such as breeding, genetic and biotechnological research, and production of raw material (Endress, 1994; Sahai et al., 2010). Micropropagation of some members of the Scrophulariaceae has been described, for example in *Digitalis lanata* (Lindemann and Luckner, 1997; Erdei et al., 1981; Müller-Uri and Diettrich, 1999), *Torenia fournieri* (Aida et al., 2000), *Bacopa monnieri* (Tiwari et al., 1998; Shrivastava and Rajani, 1999), *Verbascum thapsus* (Turker et al., 2001) and *Verbascum speciocum* (Karamian and Ghasemlou, 2013b). There are no reports showing micropropagation of *Verbascum sinuatum*. Here we describe a protocol for organogenesis and somatic embryogenesis through mature embryo culture of *Verbascum sinuatum*.

*e-mail: R_karamian@basu.ac.ir

PL ISSN 0001-5296

TABLE 1. Effect of different concentrations of NAA and BA on callus, shoot and root induction in *Verbascum sinuatum*

Growth regulator (mg l^{-1})		Callus (%)	Embryogenic callus (%)	Shoot (%)	Root (%)
BA	NAA				
0	0	0	0	33.33^{bc}	33.33^{b}
0	1.5	50^{bc}	0	0	50^{ab}
0	3	0	0	0	0
2.5	0	43.7^{c}	18.75^{c}	18.75^{c}	0
2.5	1.5	62.5^{bc}	50^{abc}	50^{abc}	33.33^{b}
2.5	3	100^{a}	62.5^{ab}	62.5^{ab}	68.5^{a}
5	0	45.8^{c}	58.33^{ab}	58.33^{ab}	12.5^{c}
5	1.5	68.7^{b}	37.5^{abc}	37.5^{abc}	12.5^{c}
5	3	100^{a}	79.16^{a}	79.16^{a}	0

Values are means of three experiments with 24 replicates per experiment. Means bearing the same letters within column do not significantly differ at $P < 0.05$.

MATERIALS AND METHODS

CALLUS INDUCTION

Plants of *Verbascum sinuatum* were collected from Hamedan Province (W Iran) from July to August 2011. Voucher specimens are deposited in the herbarium of Bu-Ali Sina University (BASU), Hamedan, Iran. Different explants including leaves, petioles and capsules were washed with tap water, surface-sterilized in 70% (v/v) EtOH and 5% sodium hypochlorite for 15 min, then rinsed three times in sterile distilled water. Mature embryos, leaves and petioles were dissected and cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with BA (0, 2.5 or 5 mg l^{-1}), NAA (0, 1.5 or 3 mg l^{-1}), 3% (w/v) sucrose and 1% (w/v) agar. Media pH was adjusted to 5.7–5.8 prior to autoclaving for 15 min at 121°C and 15 lbs/in^2 pressure. After inoculation to assure uniform and rapid growth of mature embryos, all cultures were kept at 5°C for 48 h, then incubated at 25±2°C under a 16 h photoperiod (cool white fluorescent light, 3000 lux) in a culture room. Data on explants producing calli were recorded after 4 weeks and calli were subcultured on fresh MS medium after 30 days of culture. There were 24 replicates for each explant and the experiments were done three times.

EMBRYOGENIC CALLUS PROLIFERATION

To promote multiplication of somatic embryoids and shoot bud differentiation, pieces of yellow and friable embryogenic calli produced on medium containing BA or NAA were transferred to 3 new media on day 30 of culture: (1) MS medium containing 30 g l^{-1} sucrose, 0.1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} charcoal; (2) MS medium containing 30 g l^{-1} sucrose, 1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} charcoal; or (3) MS medium containing 60 g l^{-1} sucrose, 50 mg l^{-1} CH, 0.5 mg l^{-1} Kin, 5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} charcoal. Data were recorded after 6 weeks of culture. Then the embryogenic calli along with somatic embryos in earlier stages were transferred to MS medium without plant growth regulators, to develop somatic embryos and induce shoot bud initials.

SHOOT AND ROOT INDUCTION

Shoot bud initials induced from embryogenic calli on MS medium containing BA and NAA were transferred to MS medium supplemented with 1 mg l^{-1} BA or Kin for further growth and multiplication. Data were recorded after 5 weeks. For root proliferation and plantlet regeneration, shoots with thin roots were transferred to rooting medium containing 3 mg l^{-1} NAA.

PLANT ACCLIMATIZATION

For acclimatization, healthy plantlets with well-developed roots and shoots were chosen, washed in water to remove agar, and transferred to pots containing sterilized soil and perlite (1:1). The transplanted plants were covered with clear plastic bags to maintain humidity, placed in an acclimatization chamber, and watered with 1/4 MS minerals at 4-day intervals. After 2 weeks the plastic bags were opened and the uncovered plants were maintained under natural daylight at 19–23°C in the laboratory. The survival frequency of the propagated plants was assessed at week 3 of the acclimatization process.

STATISTICAL ANALYSIS

Each triplicate experiment used 24 embryo explants or calli. Data were analyzed using SAS ver. 6.12 (SAS Institute, 1995, Cary, N. C.). All experimental

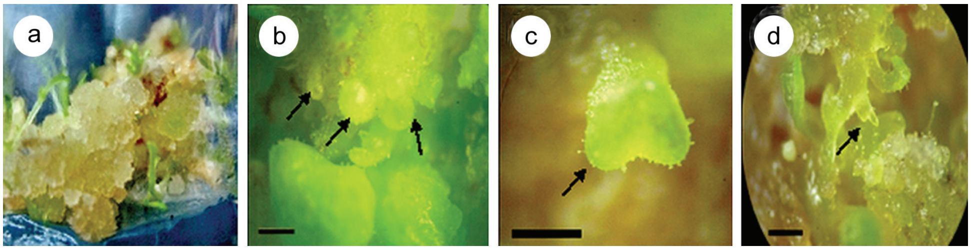

Fig. 1. Somatic embryogenesis and organogenesis in *Verbascume* sinuatum. (**a**) Nonembryogenic calli induced from mature embryo explants on MS medium containing 30 g l^{-1} sucrose, 2.5 mg l^{-1} BA and 3 mg l^{-1} NAA after 4 weeks, (**b**) Globular somatic embryos on MS medium containing 30 g l^{-1} sucrose, 5 mg l^{-1} BA and 3 mg l^{-1} NAA (arrows), (**c**) Heart-shaped somatic embryos on MS medium containing 30 g l^{-1} sucrose, 1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} charcoal (arrow), (**d**) Somatic embryos at cotyledonary stage on MS medium containing 30 g l^{-1} sucrose, 1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} charcoal after 4 weeks (arrow). Bars = 10 mm.

results were tested by ANOVA, and the significance of differences among means was tested with Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

CALLUS INDUCTION

Growth and morphogenesis in vitro are controlled by the kind and concentration of plant growth regulators (Fatima et al., 2009). The type of explant also plays a considerable role in callus induction. In *Verbascum sinuatum* the choice of explants is crucial for callus induction. Callus was induced only from mature embryos; leaf and petiole explants did not show any morphogenetic response. This result supports the idea that the internal state of explant cells is most important to the expression of somatic embryogenesis, with other conditions such as exogenous growth regulators simply enabling expression of this intrinsically determined pattern of development (Williams and Maheswaran, 1986; Zimmerman, 1993).

Callus was induced on all media supplemented with different concentrations of BA and NAA, except for growth regulator-free medium and medium with 1.5 or 3 mg l^{-1} NAA. The frequency of nonembryogenic callus initiation was highest on MS medium supplemented with 5 mg l^{-1} BA and 3 mg l^{-1} NAA or with 2.5 mg l^{-1} BA and 3 mg l^{-1} NAA. These calli were heterogeneous in appearance and showed morphogenetic potential (Tab. 1). However, calli formed in the presence of 1.5 mg l^{-1} NAA only produced adventitious roots vigorously in this medium later.

EMBRYOGENIC CALLUS INDUCTION

Somatic embryogenesis has been induced from various explants in many taxa (Williams and Maheswaran, 1986). Many factors including the choice of plant growth regulators and explants are involved in successful somatic embryogenesis (Luo and Jia, 1999). According to Yasuda et al. (1985) and Pasquale et al. (1994) somatic embryogenesis has been induced in many plant species using BA combined with 2,4-D or NAA, but induction of embryogenesis using BA alone is very rare. In this study we induced embryogenic and nonembryogenic callus using 2.5 mg l^{-1} BA alone. Embryogenic calli were observed at different concentrations of BA and NAA, not on growth regulator-free medium and not on medium with 1.5 or 3 mg l^{-1} NAA (Tab. 1). Somatic embryos were observed on yellowish green embryogenic calli after subculturing them in the same fresh medium. Then the calli turned green, hard, and compact, while their peripheral portions remained yellowish green and friable. Somatic embryo maturation was observed after 4 weeks of culture (Fig. 1). Shoots and roots were induced from explants in some media containing BA and NAA. Root induction was promoted by increasing the NAA level, and reduced strongly by increasing BA in the medium. However, no plantlet regeneration was achieved on these media.

EMBRYOGENIC CALLUS PROLIFERATION

The growth of yellowish white and friable embryogenic calli was promoted by transferring them to new MS media (Fig. 2). Among the three media tested, MS medium containing 30 mg l^{-1} sucrose, 1 mg l^{-1} or 0.1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} charcoal were more effective in promoting embryonic calli (Tab. 2). Our results are consistent with reports emphasizing the role of auxin in embryogenesis induction. In some plant species a high level of 2,4-D (up to 2 mg l^{-1}) is needed to enhance embryogenic callus production (Nagarajan et al., 1986; Nolan et al., 1989; Luo and Jia, 1999). Compact yellowish white calli spontaneously formed vigorous embryoids and the somatic embryos passed through each of the typical devel-

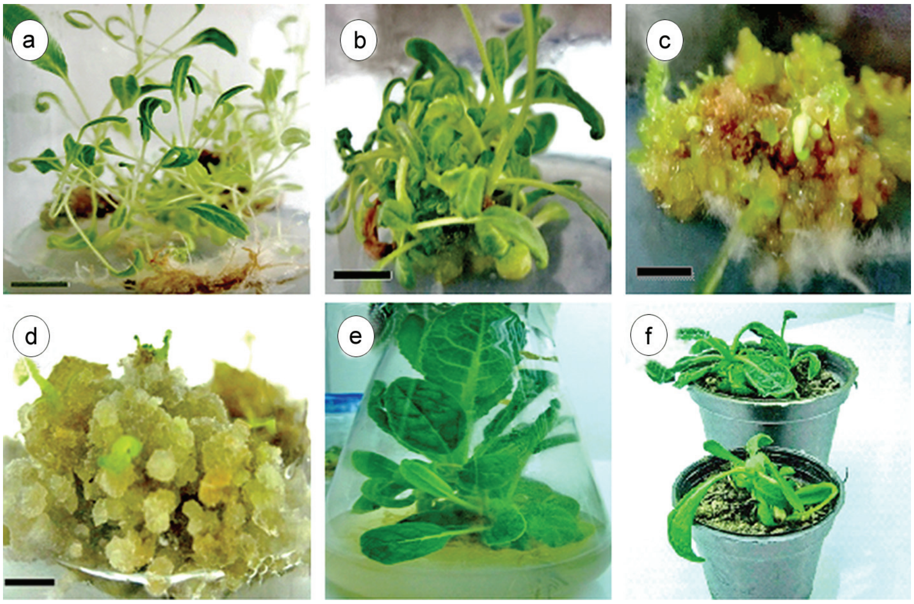

Fig. 2. Shoot multiplication and plantlet regeneration in *Verbascum sinuatum*. (**a, b**) Shoot multiplication on MS medium containing 5 mg l^{-1} BA and 3 mg l^{-1} NAA (**a**) or 2.5 mg l^{-1} BA and 3 mg l^{-1} NAA (**b**) after 6 weeks, (**c, d**) Embryogenic callus proliferation on MS medium supplemented with 0.1 (**c**) or 1 mg l^{-1} 2,4-D (**d**) 6 weeks after transferring from MS medium containing 5 mg l^{-1} BA and 1.5 mg l^{-1} NAA, (**e**) Multiple shoot proliferation 5 weeks after transferring to MS medium containing 1 mg l^{-1} BA, (**f**) Acclimatization of plantlets. Bars = 10 mm.

opmental stages. MS medium containing 60 mg l^{-1} sucrose, 0.5 mg l^{-1} Kin, 5 mg l^{-1} 2,4-D, 50 mg l^{-1} CH and 0.5 mg l^{-1} charcoal had no significant effect on embryogenic callus proliferation (Tab. 2).

SHOOT AND ROOT PROLIFERATION

Torregrosa and Bouquet (1996) noted that shoot regeneration could be obtained in the presence of BA alone but that auxin could enhance the organogenic response. In order to obtain a high frequency of adventitious shoot regeneration we adjusted the concentrations and combinations of auxins and cytokinins. Shoots have been regenerated directly from explants in numerous species. Plants that propagate adventitiously by conventional means may be proliferated rapidly in vitro not only from the organs commonly used as explant sources but also from other tissues not normally associated with vegetative reproduction (Meins, 1986).

In the present study, nodular structures that proliferated in the presence of 2,4-D differentiated into organized structures of shoot buds. Shoot buds grew further and became well-developed shoots after 5 weeks in the presence of BA or Kin, in comparison to primary medium containing both of BA and NAA (Fig. 2). BA proved more effective than Kin; the number of shoots was highest on medium with 1 mg l^{-1} BA (Tab. 3). *Verbascum sinuatum* showed superior rhizogenesis potential. Plantlets regenerated on medium with 5 mg l^{-1} BA. Similar results were reported for shoot induction from *Verbascum thapsus* stem explants (Caruso, 1971; Turker et al., 2001). Our results are consistent with those of Skoog and Miller (1957). They stated that a low ratio of auxin to cytokinin induces shoots, while the reverse induces roots. The in vitro culture protocol we established for *Verbascum sinuatum* can provide material for future physiological and biochemical studies, for example those aimed at developing extraction and analytical procedures for its active ingredients.

PLANT ACCLIMATIZATION

Well-developed and rooted plantlets were transferred to sterile soil with perlite for acclimatization and covered with plastic bags to ensure high humidity around the plants. Two weeks later the plastic bags were removed and the plants were transferred to ex vitro conditions. At week 3 of acclimatization, all of the plantlets survived and showed normal growth (Fig. 2).

CONCLUSION

Many medicinal plant species are propagated in vitro because they do not respond well to conventional methods of propagation. An in vitro culture

TABLE 2. Effects of new media on embryogenic callus and shoot bud induction 6 weeks after transferring from MS medium containing NAA and BA in *Verbascum sinuatum*

Primary medium		1 mg l^{-1} 2,4-D		0.1 mg l^{-1} 2,4-D		0.5 mg l^{-1} Kin + 5 mg l^{-1} 2, 4-D + 50 mg l^{-1} CH	
BA (mg l^{-1})	NAA (mg l^{-1})	Embryogenic callus (%)	Shoot (%)	Embryogenic callus (%)	Shoot (%)	Embryogenic callus (%)	Shoot (%)
2.5	0	88.88[a]	34.44[b]	100[a]	66.4[a]	33.44[a]	0
2.5	1.5	100[a]	0	34.44[c]	63.33[a]	0	0
2.5	3	33.1[b]	0	33.2[c]	35.3[b]	0	0
5	0	34.42[b]	0	0	66.4[a]	0	0
5	1.5	44.44[b]	0	62.33[b]	65.2[a]	0	0
5	3	33.33[b]	66.44[a]	0	34.3[b]	0	0

Values are means of three experiments with 24 replicates per experiment. Means bearing the same letters within column do not significantly differ at P < 0.05.

TABLE 3. Effect of BA and Kin on shoot and root regeneration after 5 weeks in *Verbascum sinuatum*

Primary medium (mg l^{-1})		BA (1 mg l^{-1})			Kin (1 mg l^{-1})		
BA (mg/l)	NAA (mg/l)	Shoot (%)	Root (%)	Plantlet (%)	Shoot (%)	Root (%)	Plantlet (%)
0	0	100	34.44[b]	0	100[a]	43.33[a]	0
2.5	0	100	0	0	66.44[b]	0	0
2.5	1.5	100	34.44[b]	0	100[a]	0	0
2.5	3	100	33.44[b]	0	100[a]	48.33[a]	0
5	0	100	76.66[a]	65.22[a]	100[a]	48.33[a]	65.33[a]
5	1.5	100	77.77[a]	0	66.44[b]	48.33[a]	0
5	3	100	34.44[b]	0	100[a]	0	0

Values are means of three experiments with 24 replicates per experiment. Means bearing the same letters within column do not significantly differ at P < 0.05.

protocol is important when a source of plant material free of adulterated bulk material or material free of pesticide residues or disease infection is needed for medical purposes. Here we reported a procedure for successful in vitro regeneration of the medicinal plant *Verbascum sinuatum* through mature embryo culture, for the first time in this species. Embryogenic callus was induced on MS medium supplemented with different concentrations of NAA and BA. Further growth and maturation of somatic embryos were not achieved with this hormonal combination; shoot and root induction was observed in the presence of NAA and BA but no regenerated plantlet was obtained. NAA alone was more effective for root induction, while BA was better for shoot regeneration. Our results confirm the value of 2,4-D for embryogenic callus proliferation and somatic embryo maturation in *Verbascum sinuatum*, but shoot bud initials did not differentiate further in the presence of 2,4-D. Cultures containing BA or Kin alone showed multiple shoot induction and plantlet regeneration, as compared to medium containing BA and NAA.

AUTHORS' CONTRIBUTION

FG obtained in vitro cultures, performed the experiment work, wrote draft of the manuscript; RK participation in all experiments; data analysis; interpretation of the results; critical revision of the manuscript. The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

We thank Ramtin Pakzad for valuable assistance in the laboratory. This research was funded by grants from Bu-Ali Sina University.

REFERENCES

AFIFI MSA, AHMAD MM, PEZZUTO JM, and KINGHORN D. 1993. Cytotoxic flavonolignans and flavones from *Verbascum sinaiticum* leaves. *Phytochemistry* 34: 839–841.

AHMED B, AL-REHAILY AJ, AL-HOWIIRNY TA, EL-SAYED KA, and AHMAD MS. 2003. Scropolioside-D2 and harpagoside-B: two new iridoid glycosides from *Scrophularia deserti* and their antidiabetic and anti-inflammatory activity. *Biological and Pharmaceutical Bulletin* 26: 462–467.

AIDA R, KISHIMOTO S, TANAKA Y, and SHIBATA M. 2000. Modification of flower color in torenia (*Torenia fournieri* Lind.) by genetic transformation. *Plant Science* 153: 33–42.

AKDEMIR ZS, TATLI II, BEDIR E, and KHAN IA. 2004. Iridoid and phenylethanoid glycosides from *Verbascum lasianthum*. *Turkish Journal of Chemistry* 28: 227–234.

AKDEMIR ZS, TATLI II, BEDIR E, and KHAN IA. 2005. Two new iridoid glycosides from *Verbascum salviifolium* Boiss. *Zeitschriftfur Naturforschung* B 60b: 113–117.

AMIRNIA R, KHOSHNOUD H, ALAHYARY P, GHIYASI M, TAJBAKHSH M, and VALIZADEGAN O. 2011. Antimicrobial activity of *Verbascum speciosum* against three bacteria strains. *Fresenius Environmental Bulletin* 20: 690–693.

BAYTOP T. 1999. *Therapy with Medicinal Plants in Turkey (Past and Present)*. 2nd [ed.], Nobel Tip Kitabevleri Ltd, 334. Istanbul.

CARUSO JN. 1971. Bud formation in excised stem segments of *Verbascum thapsus* L. *American Journal of Botany* 58: 429–431.

DRANDAROV K, and HAIS IM. 1997. Separation of EZ isomeric macrocyclic spermine alkakoids of *Verbascum pseudonobile* and *Verbascum phoeniceum* and of their derivatives using thin-layer chromatography. *Journal of Chromatography* 724: 416–423.

ENDRESS R. 1994. *Plant Cell Biotechnology*. 1st [ed.], 46–83. Berlin Springer-Verlag, Heidelberg.

ERDEI I, KISS Z, and MALIGA P. 1981. Rapid clonal multiplication of *Digitalis lanata* in tissue culture. *Plant Cell Reports* 1: 34–35.

FATIMA Z, MUJIB A, FATIMA S, ARSHI A, and UMAR S. 2009. Callus induction, biomass growth, and plant regeneration in *Digitalis lanata* Ehrh.: influence of plant growth regulators and carbohydrates. *Turkish Journal of Botany* 33: 393–405.

HARTLEB I, and SEIFERT K. 1994. Sangarosaponin-D a triterpenoid saponin from *Verbacsum songaricum*. *Phytochemistry* 35: 1009–1011.

HUBER-MORATH A. 1978. *Verbascum* L. In: Davis PH [ed.], *Flora of Turkey and the East Aegean Islands*, 6, 461–600. Edinburgh University Press.

JUDD WS, CAMPBELL CS, KELLOGG EA, and STEVENS PF. 2002. *Plant Systematics: A Phylogenetic Approach*. 576. Sinauer, Sunderland.

KARAMIAN R, and GHASEMLOU F. 2014. Study of saponin contents from the aerial parts and roots of three *Verbascum* L. species. *Iranian Journal Biology* 4: 23–30.

KARAMIAN R, and GHASEMLOU F. 2013a. Total phenolics content, antioxidant and antibacterial activities of three *Verbascum* species from Iran. *Journal of Medicinal Plants and By-products* 1: 43–51.

KARAMIAN R, and GHASEMLOU F. 2013b. Plant regeneration via somatic embryogenesis and organogenesis in *Verbascum speciosum* Schard. *Journal of Cell and Molecular Research* 4 (2): 81–88.

KARAVELIOGULLARI FA, DURAN A, and HAMZAOGLU E. 2004. *Verbascum tuna-ekimii* (Scrophulariaceae), a new species from Turkey. *Annales of Botanici Fennici* 41: 222–227.

KONOSHIMA T, TAKASAKI M, TOKUDA H, and NISHIMO H. 2000. Cancer chemo-preventive activity of an iridoid glycoside, 8-acetylharpagide from *Ajuga decumbens*. *Cancer Letters* 157: 87–92.

LINDEMANN P, and LUCKNER M. 1997. Biosynthesis of pregnane derivates in somatic embryos of *Digitalis lanata*. *Phytochemistry* 46: 507–13.

LUO JP, and JIA JF. 1999. Callus induction and plant regeneration from hypocotyl explants of the forage legume *Astragalus adsurgens*. *Plant Cell Reports* 17: 567–570.

MEINS FJR. 1986. Determination and morphogenetic competence in plant tissue culture. In: YEOMAN MM [ed.], *Plant Cell Culture Technology*, 23, 7–25. Botanical monographs. Blackwell Scientific Publications, Oxford.

MÜLLER-URI F, and DIETTRICH B. 1999. Kultivierung proembryogener Massen von *Digitalis lanata* im Mini Perm Bioreaktor. *In Vitro News* 3: 4.

MURASHIGE T, and SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

NAGARAJAN P, MCKENZIE JS, and WALTON PD. 1986. Embryogenesis and plant regeneration of *Medicago* spp. in tissue culture. *Plant Cell Reports* 8: 279–281.

NOLAN KE, ROSE RJ, and GORST JR. 1989. Regeneration of *Medicago truncatula* from tissue culture: increased somatic embryogenesis using explants from regenerated plants. *Plant Cell Reports* 8: 278–281.

PASQUALE FD, CARIMI F, and CRESCIMANNO FG. 1994. Somatic embryogenesis from styles of different cultivars of *Citrus limon* (L.) Burm. *Australian Journal of Botany* 42(5): 587–594.

SAHAI A, SHAHZAD A, and ANIS M. 2010. High frequency plant production via shoot organogenesis and somatic embryogenesis from callus in *Tylophora indica*, an endangered plant species. *Turkish Journal of Botany* 34: 11–20.

SENATORE F, RIGANO D, FORMISANO C, GRASSIA A, BASILE A, and SORBO S. 2007. Phytogrowth-inhibitory and antibacterial activity of *Verbascum sinuatum*. *Fitoterapia* 78: 244–247.

SEZIK E, YESILADA E, HONDA G, TAKAISHI Y, TAKEDA Y, and TANAKA T. 2001. Traditional medicine in Turkey X. Folk medicine in Central Anatolia. *Journal of Ethnopharmacology* 75: 95–115.

SHARIFNIA F. 2007. Notes on the distribution and taxonomy of *Verbascum* in Iran. *Iranian Journal of Botany* 31(1): 30–32.

SHRIVASTAVA N, and RAJANI M. 1999. Multiple shoot regeneration and tissue culture studies on *Bacopa monnieri* (L.) Pennell. *Plant Cell Reports* 18: 919–923.

SKOOG F, and MILLER CO. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposia of the Society for Experimental Biology* 11: 118–130.

STEVENSON PC, SIMMONDS MSJ, SAMPSON J, HOUGHTON PJ, and GIRCE P. 2002. Wound healing activity of acylated iridoid glycosides from *Scrophularia nodosa*. *Phytotherapy Research* 16: 33–35.

TATLI II, and AKDEMIR ZS. 2004. Chemical constituents of *Verbascum* L. species. *Journal of Pharmaceutical Sciences (FABAD)* 29 (2): 93–107.

TATLI II, AKDEMIR ZS, BEDIR E, and KHAN IA. 2003. 6-O-α-L-Rhamnopyranosyl catalpol derivative iridoids from *Verbascum cilicicum*. *Turkish Journal of Chemistry* 27: 765–772.

TATLI II, AKDEMIR ZS, BEDIR E, and KHAN IA. 2004. Saponin, iridoid, phenylethanoid and monoterpene glycosides from *Verbascum pterocalycinum* var. Mutense. *Turkish Journal of Chemistry* 28: 111–122.

TIWARI V, SINGH BD, and TIWARI KN. 1998. Shoot regeneration and somatic embryogenesis from different explants of *Brahmi* [*Bacopa monnieri* (L.) Wettst.]. *Plant Cell Reports* 17: 538–543.

TORREGROSA L, and BOUQUET A. 1996. Adventitious bud formation and shoot development from in vitro leaves of Vitis × *Muscadinia* hybrids. *Plant Cell Tissue and Organ Culture* 45: 245–252.

TURKER AU, and CAMPER ND. 2002. Biological activity of common mullein, a medicinal plant. *Journal of Ethnopharmacology* 82: 117–125.

TURKER AU, CAMPER ND, and GUREL E. 2001. In vitro culture of common mullein (*Verbascum thapsus* L.). *In vitro Cell Development Biology – Series Plant* 37: 40–43.

TUZLACI E, and EROL MK. 1999. Turkish folk medicinal plants. Part II. Egirdir (Isparta). *Fitoterapia* 70: 593–610.

VALDES B. 1987. Scrophulariaceae. In: Valdes BS, Talveraand E, Fernandez G [eds.], *Flora Vascular de Andalucia Occidental*, vol. 2, 486–547. Ketres, Barcelona.

VIJAYAVITTHAL TM, KANWAL R, AMIYA PB, RAGINI S, ANJU P, LALIT MT, and VISHWA MLS. 1998. Studies on the profile of immunostimulant activities of modified iridoid glycosides. *Bioorganic and Medicinal Chemistry* 6: 605–611.

WILLIAMS EG, and MAHESWARAN G. 1986. Somatic embryogenesis: factors influencing coordinated behavior of cells as an embryogenic group. *Annals of Botany* 57: 443–462.

YASUDA T, FUJII Y, and YAMAGUCHI T. 1985. Embryogenic callus induction from *Coffea arabica* leaf explants by BA. *Plant Cell Physiology* 26: 595–597.

ZIMMERMAN JI. 1993. Somatic embryogenesis: a model for early development in higher plants. *Plant Cell* 5: 1411–1423.

ZOHARY M. 1973. *Geobotanical Foundations of Middle East*, 307–329. Hebrew University, Jerusalem, Israel.