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Full Length Research Paper

Plant regeneration of *Senecio hypochionaeus* var. *argaeus*

Hatice Çölgeçen

Department of Biology, Faculty of Arts and Science, Zonguldak Karaelmas University, 67100 Incivez, Zonguldak, Turkey. E-mail: haticecolgecen@gmail.com or colgecen@karaelmas.edu.tr.

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Senecio **genus includes taxa that can be used for agricultural, pharmacological, physiological and biochemical studies, on account of their different pyrrolizidine alkaloids (PAs).** ***Senecio hypochionaeus*** **var.** ***argaeus*** **is an endemic species in Turkey. Successful callus production was achieved in all the N^6-benzyladenine (BA) and naphthaleneacetic acid (NAA) experiments in the root explants. The highest level of shoot generation in the apical meristem explants was observed in Murashige and Skoog medium that contained 1 mg/l BA and 1 mg/l NAA. The best root formation was obtained from 8 mg/l indolebutyric acid. Young rooted shoots were transplanted to pots with sterile garden soil, and then grown in an acclimatization room.**

Key words: Asteraceae, plant regeneration, *Senecio hypochionaeus* var. *argaeus.*

INTRODUCTION

Senecio spp. grow in poor sandy dune soils and at the roadside, but are also found as noxious weeds in pastures, where they cause acute and chronic liver damage when ingested by cattle. The use of *Senecio* spp. in traditional human medicine has carcinogenic effects. These effects are caused by pyrrolizidine alkaloids (PAs), which are regarded as part of the defense mechanism of the plant against herbivores. PAs production in *Senecio* spp. takes place in the root and is related to root growth. In *Senecio* spp., 124 different PAs structures have been described and in a single species such as *Senecio jacobaea* L., up to 14 PAs are found. Researchers indicated that herbivores are sometimes attracted by PAs and sequester them for their own defense. Adapted insects store plant-derived PAs, preferably as N oxides. N oxidation is believed to be a detoxification mechanism. The target of PAs in mammals is cytochrome P450. *In vitro* assays have demonstrated that PAs can interfere with neuroreceptors and protein synthesis (Hol and Van veen, 2002; Hol et al., 2003). In addition to their use for the treatment of wounds, abscesses, eczema and scabies, leaves of *Senecio ambavilla* Pers. also have inhibitory effects against herpes simplex virus type 1 and poliovirus type 2 (Fortin et al., 2002). PAs extract from *Senecio brasiliensis* var. *brasiliensis* (Spreng.) inflorescences has a significant anti-ulcer effect in selected models (Toma et al., 2004). The juice obtained from the roots of *Senecio vulgaris* L. can be rubbed on skin areas with eczema (Uzun et al., 2004).

Regeneration studies have been carried out worldwide in diverse *Senecio* species. Callus induction and plant regeneration have been achieved from *Senecio cineraria* cv. Silver Dust leaves (Gong et al., 2004), *Senecio* x *hybridus* petals (Takeo et al., 1998), mesophyll protoplasts (Pillai et al., 1990), pedicel and receptacles (XiaoYing et al., 2005). Direct somatic embryogenesis has been achieved from leaves of *Senecio* x *hybridus* (Malueg et al., 1994). *Senecio hypochionaeus* var. *argaeus* is an endemic species in Turkey. It is distributed in Erzincan, Giresun, Gümüşhane and Kayseri provinces. It is classified in the lower risk category in the Red Data Book of Turkish Plants (Ekim, 2000). No study has been done on tissue culture of endemic *Senecio* taxa in Turkey. Furthermore, there have been only a few pharmacological studies demonstrating the presence of PAs in endemic *Senecio* taxa (Noorwala et al., 2000; Uzun et al., 2004). *Senecio* taxa are not very important medicinal plants. However, PAs, particularly those within the roots of *Senecio*, can be used pharmacologically as insecticides and against herbivores. For this reason, the adaptation of *Senecio* to tissue culture medium is important for PAs production. During our regeneration study, S. *hypochionaeus* var. *argaeus* was subjected to tissue culture, and the study was aimed at contributing to

Table 1. Callus response (%) of *S. hypochionaeus* var. *argaeus* explants in three MS media (means ± SE).

Growth regulator (mg/l)		MS			
BAP	NAA	Apical meristem	Cotyledon	Leaf	Root
0.5	1	48.82 ±2.89[a]WY	33.82±0.81[a] YG	42.36±1.14[a]YG	84.56±0.00[a] WY
0.8	1	52.98±1.55[a]WY	16.91±0.75[a] YG	46.15±1.28[a]YG	77.09±3.8[a]WY
1	1	67.65±1.91[a]WY	15.81±0.75[a] YG	63.69±0.85[b]YG	84.56±0.00[a]WY

Means followed by the same letter were not significantly different by Duncan multiple comparison test between different concentrations of BAP. W, white callus; Y, yellow callus; G, green callus.

the conservation of endemic species.

MATERIALS AND METHODS

Seeds of *S. hypochionaeus* Boiss. var. *argaeus* (Boiss. & Bal.) Matthews (Matthews, 1975) were collected in July 2005 from Kayseri, Erciyes Mountain, northeast of Perilikartın and overlooking Lifos at an altitude of 2245 m. 45 day-old aseptic seedlings were used as an explant source. The pappi of the seeds were removed. Seeds were sterilized in 96% ethanol for 1 min and then transferred to 15% sodium hypochlorite solution for 10 min (commercial sodium hypochlorite was used in the sterilization process). Then, the seeds were rinsed three times in autoclaved distilled water. One group of seeds was separated from their pappus region by cutting, and the other group of seeds was left intact. Pappus-cut and intact seeds were placed in hormone-free Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar. Apical meristem (1 mm), cotyledon (whole, and as two fragments), leaves (5 mm) and roots (5 to 7 mm) of aseptically grown seedlings provided explant tissues. The explants were cultured in Petri dishes (100 × 15 mm).

Plant regeneration

MS medium (3% sucrose and 0.8% agar) was used for the *in vitro* organogenesis. Naphthaleneacetic acid (NAA; 0.1 mg/l) with 0.5, 0.8 or 1 mg/l N^6-benzyladenine (BA) were used in three different compositions of MS medium for shoot formation (Table 1). All media were adjusted to pH 5.8 before autoclaving. The calli with shoots were subcultured in the same medium every 4 weeks, immediately after darkening of the calli. All the samples were incubated at 23 ± 1°C with a 16/8-h photoperiod (irradiance of 42 µmol/m^2/s was provided by cool-white fluorescent tubes).

The shoots (1 to 1.5 cm) obtained from MS medium were transferred into fresh medium that contained 8 mg/l indolebutyric acid (IBA) and 9 mg/l NAA for rooting in jars (100 × 200 mm) under aseptic conditions, and incubated at 22 to 24°C with a 16/8-h photoperiod (irradiance of 42 µmol/m^2/s was provided by cool-white fluorescent tubes).

Acclimatization of the rooted plantlets was achieved via removing the jar lids gradually with increased duration throughout a week in a growth chamber. Plantlets were transplanted into sterilized garden soil. The humidity ratio of the growth chamber was gradually decreased from 80 to 55 to 50%.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and the differences among means were compared by Duncan's multiple-range test (Duncan, 1955). Two experimental rooting hormone applications were compared using a paired Student's *t* test. Each treatment was replicated three times and arranged in a completely randomized design. The data given in percentages were subjected to arcsine transformation (Snedecor and Cochran, 1967) before statistical analysis.

RESULTS AND DISCUSSION

The first calli were observed on day 10 in different tissues; the apical meristem, cotyledon, root and leaf explants. Off-white, yellow and green calli were observed in all the explants (Figure 1a). Except for cotyledons, 1 mg/l BAP and 1 mg/l NAA concentration produced the most sucessful results among all the explants. The low callus response in cotyledons can be attributed to the weakening of cotyledons in the 45-day-old aseptic seedlings while, in our study, the highest frequency of callus production from *S. cineraria* (*S. bicolor*) cv. Silver Dust leaves was obtained from MS medium with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) + 1 mg/l BA + 0.1 mg/l NAA. The highest frequency of callus production was found previously in roots, apical meristem and leaves in descending order (Gong et al., 2004). Callus response in roots was more successful as compared to that in other explants (Figure 1b). A high frequency of callus production was achieved with all concentrations of BAP. The most successful callus production in the apical meristem and leaves was obtained from 1 mg/l BAP and 1 mg/l NAA, while 0.5 mg/l BAP and 1 mg/l NAA provided the best results in cotyledons (Table 1). Callus production and plant regeneration have was obtained from pedicel and receptacles in *Senecio* x *hybridus* (XiaoYing et al., 2005). The media of 3/4 MS + 1 mg/l BA + 1 mg/l NAA and 3/4 MS + 1 mg/l BA had a higher frequency of callus production in receptacles as compared to pedicels (XiaoYing et al., 2005) whereas, the highest amount of calli were observed in the roots in our study. The PAs in the roots of the taxa of this species are emphasized with respect to the different secondary metabolites (Topel et al., 1987; Tripathi and Tripathi 2003). Hence, callus production from the roots will provide data for the analysis and production of these compounds.

Shoots were regenerated from the white and yellow calli in the apical meristem explants in all media. From the third week onwards and in subculture, shoots were formed from the calli through indirect organogenesis.

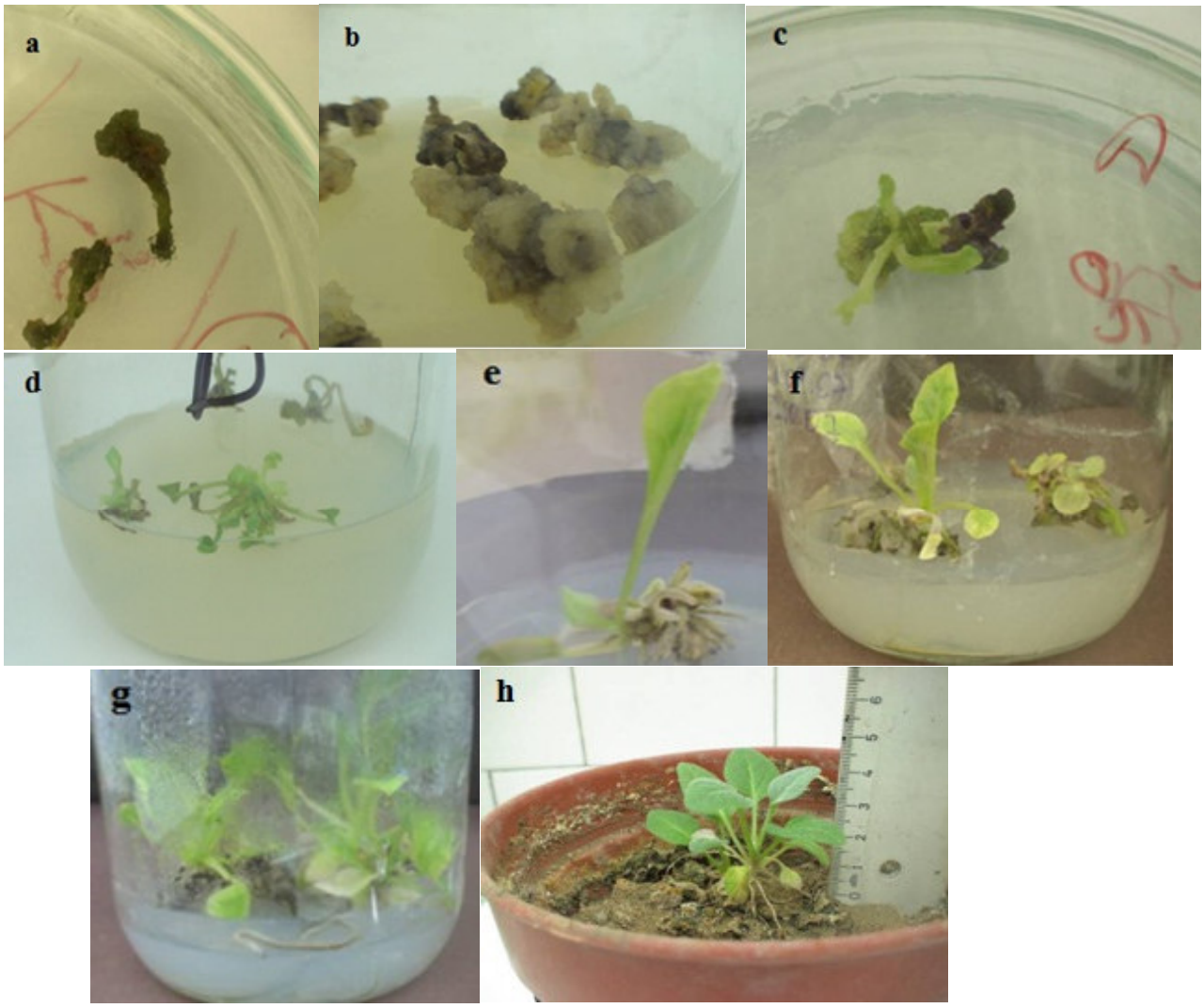

Figure 1. *In vitro* regeneration of S. *hypochionaeus var. argaeus*. A, Green callus in hypocotyl explants; b, White, yellow callus in root explantsp; c and d, shoot regeneration from apical meristem explants cultured in MS medium containing 1 mg/l BAP and 1 mg/l NAA; e to g, rooting of generated shoots in medium containing 8 mg/l IBA; H, regenerated plants in pot containers including garden soil under growth room conditions.

While no shoots were produced from cotyledon and root explants, one or two shoots were regenerated from leaf explants. While very few shoots were generated from the apical meristem in 0.5 or 0.8 mg/l BAP + 1 NAA mg/l medium, the most successful shoot formation was achieved in medium with 1 mg/l BAP + 1 NAA mg/l (Figure 1c and d). The number of shoots generated from the leaves was low. The most appropriate concentration for shoot formation in this species was 1 mg/l BAP + 1 NAA mg/l. Shoot regeneration from the leaves of *S. cineraria* (*S. bicolor*) cv. Silver Dust was achieved in MS medium with 0.5 mg/l BA + 0.1 mg/l NA (Gong et al., 2004). In our study, shoots were produced from apical meristem and leaves in MS medium with 1 mg/l BA + 1 mg/l NAA. The amounts of IAA and IBA that were used in the cultures of *Senecio* x *hybridus* were the same as the amounts of NAA and BA in our study (Takeo et al., 1998). In a study on *Senecio cruentus*, experiments were conducted with 4.5 to 13.5 µM 2,4-D and 1.5 to 4.5 µM BA, kinetin or thidiazuron for direct somatic embryogenesis (Eunyoung et al., 2005). Successful results were achieved in the explants of cotyledons and hypocotyls, which differed from our study. The combination of NAA and BA led to good results for study. In another study on *Senecio* x *hybridus*, a 90% plant renegeneration rate was achieved in the pedical calli in 3/4 MS medium without any thidiazuron, which differed from our study (XiaoYing et al., 2005). Normal leaf formation was observed in all the shoots (Table 2).

The regenerated shoots were rooted in MS medium

Table 2. Effect of BAP on shoot induction (%) from calli of apical meristems and leaves in MS media (means ± SE).

Growth regulator (mg/l)		MS	
BAP	**NAA**	**Apical meristem**	**Leaf**
0.5	1	28.91 ± 0.41[a]	0.00 ± 0.02
0.8	1	37.91 ± 0.00[a]	5.77 ± 0.00[a]
1	1	79.36 ± 0.58[b]	7.39 ± 0.13[a]

Means followed by the same letter were not significantly different using the Duncan multiple comparison test between different concentrations of BAP.

with 8 mg/l IBA and 9 mg/l NAA within 7 and 10 days, respectively. The highest rooting percentage (64.78%) was achieved in MS medium with 8 mg/l IBA (Figure 1e to g). A rooting percentage of 52.50% was obtained in the MS medium with 9 mg/l NAA. After rooting, all the seedlings with a minimum of 10 to 15 leaves were transferred to pots. These plants continued to grow in growth chamber. The success rate was 80% under the acclimatization conditions. The acclimatized plants showed the same morphology as the *S. hypochionaeus* var. *argaeus* plants used in this work (Figure 1h).

The shoots regenerated from the leaves of *S. cineraria* (*S. bicolor*) cv. Silver Dust were rooted in MS medium with 0.01 mg/l NAA (Gong et al., 2004). In our study, a high level of IBA was included in the MS medium for rooting. As for rooting performance, the shoots in *Senecio* x *hybridus* were rooted in MS medium with 1/2 MS + 0.2 mg/l IBA after 10 days (Gong et al., 2004). In our study, the shoots were rooted in MS medium with 8 mg/l IBA after 7 days (XiaoYing et al., 2005).

In conclusion, this study is the first to achieve plant regeneration of *S. hypochionaeus* var. *argaeus*. The study contributes to the conservation of an endemic species and this *in vitro* propagation protocol can provide plant material for future pharmacological, physiological and biotechnological studies.

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