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Micropropagation of *Degenia velebitica* (Deg.) Hay., a Croatian Endemic Plant Species

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S u m m a r y

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The micropropagation possibility for *Degenia velebitica* (Deg.) Hay., a Croatian endemic plant species was investigated. Shoots originated from aseptically germinated seeds collected on natural habitats were used for culture initiation. The highest multiplication rate of *Degenia velebitica* shoots (5.2 shoots per explant) was achieved on half strength MS medium supplemented with 2.9 μM gibberellic acid and 0.5 μM 6-benzylaminopurine (BA). Excised shoots (2-4 cm) were rooted on the same basal medium with addition of 4.9 μM indole-3-butyric acid (IBA). Rooted plantlets were successfully transferred to potting soil and acclimatized to outdoor conditions.

Introduction

Degenia velebitica (Deg.) Hay. (*Brassicaceae*) is a rare and endangered Croatian endemic plant species. The small populations of *Degenia velebitica* grow only on calcareous rocks in a geographically limited area of middle and southern part of mountain Velebit. Since there is a risk of its extinction due to a destruction of their natural habitats and uncontrolled collecting by botanists, florists and tourists, *Degenia velebitica* has become under the protection. In previous papers mainly morphological, phytocoenological and ecological studies of that species were described (MAYER 1981). Although until today there has been some reports on in vitro culture of Croatian endemic plant species, e.g. *Fibigia triquetra* (KOSTOVIĆ-VRANJEŠ 1995, PEVALEK-KOZLINA & al. 1997) and *Centaurea*

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ragusina (PEVALEK-KOZLINA 1998), there are no reports on micropropagation of *Degenia velebitica*. Therefore, the aim of this study was to develop methods for in vitro clonal multiplication, rooting and acclimatization of that rare Croatian endemic species which will contribute to the species as well as to the biodiversity conservation.

Materials and Methods

Shoots originated from 20 to 25-day-old seedlings grown in in vitro conditions from seeds of *Degenia velebitica* collected in natural habitats were used as initial explants.

Sterilization of seeds was successively carried out with 2% (W/v) water solution of a chlorine product Izosan-G (99% sodium dichloroisocyanurate dihydrate, a commercial product of Pliva, Zagreb) for 5 min and then, after three sterile distilled water rinses (5 min each), with 6% solution of hydrogen peroxide followed by three sterile distilled water washes, each one lasting 5 min.

Single explants were inoculated in test tubes (30 × 120 mm) filled with 15 ml of agar nutrient medium. After inoculation, test tubes were capped with cotton plugs and aluminium foil. Basal medium contained MS (MURASHIGE & SKOOG 1962) mineral salts with full or half concentration of macroelements, 100 mgl^{-1} myo-inositol, 0.4 mgl^{-1} thiamine HCl, 2.9 μM gibberellic acid (GA_3), 30 gl^{-1} sucrose and 8 gl^{-1} agar. Various concentrations of 6-benzylaminopurine (BA), indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) were added to the basal medium according to the experimental objectives. The pH value of media was adjusted to 7.0 before autoclaving at 118 kPa and 120 °C for 15 minutes. The cultures were incubated at 22±2 °C under a 16 hour photoperiod (40 W fluorescent light, 80 $\mu Em^{-2}s^{-1}$).

Shoot number was estimated after 8 weeks in culture through five subcultures on at least 24 explants per medium. Statistical analysis was performed using the χ^2 test.

Results and Discussion

The sterilization procedure for seeds was satisfactory. After 4-week incubation the percentage of sterile cultures was 81.0 and the germination of seeds was relatively high (64%). After 20 to 25-days incubation, when the length of seedlings reached 2-4 cm, they were separated from primary roots and transferred to the multiplication medium (MS or 1/2 MS) supplemented with 30 gl^{-1} sucrose, 8 gl^{-1} agar and 2.9 μM GA_3 with addition of three different BA concentrations (0.2, 0.5 and 1.0 μM) or without BA.

The highest multiplication rate of *Degenia velebitica* shoots was achieved on 1/2 MS medium with addition of 0.5 μM BA (5.2 shoots per explant). Satisfactory multiplication was obtained on 1/2 MS medium with 1.0 μM BA also, but the developed shoots were shorter and had smaller leaves. The highest multiplication rate of *Degenia velebitica* shoots was significantly lower than *Fibigia triquetra* shoot multiplication (9.2 shoots per explant) on the same medium composition (PEVALEK-KOZLINA & al. 1997), although shoot number of *Fibigia triquetra* was estimated after 4 weeks and for *Degenia velebitica* after 8 weeks in culture. The multiplication rate of *Degenia velebitica* showed seasonal variability and it was higher in spring and summer period. The gradual rises in shoot number

obtained through subculturing, as it has been described for some other species (SAUER & al. 1985, BRANDT 1992, PEVALEK-KOZLINA & al. 1997, PEVALEK-KOZLINA 1998), was not observed in *Degenia velebitica* cultures (Fig. 1).

The shoot multiplication on media supplemented with 0.2 μM BA was not satisfactory, although it was slightly but not significantly higher when half instead full concentration of MS macroelements was used. The shoots inoculated on media without BA did not grow at all and eventually they died (Fig. 1). The same was described for *Fibigia triquetra* (PEVALEK-KOZLINA & al. 1997) and *Centaurea ragusina* (PEVALEK-KOZLINA 1998) cultures.

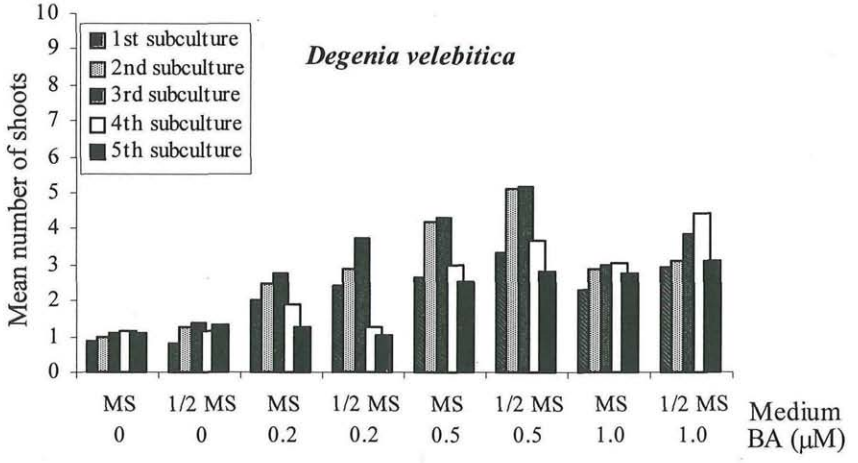

Fig. 1. The effect of different BA and MS macroelements concentrations on *Degenia velebitica* shoot multiplication through five subcultures.

Although for the culture of most *Brassicaceae* species full strength MS medium was recommended (GEORGE & SHERRINGTON 1984), we obtained better multiplication of *Degenia velebitica* shoots when inoculated on media containing half strength of macroelements. These results are corresponding to those of PEVALEK-KOZLINA & al. 1997 in culture of *Fibigia triquetra* and PEVALEK-KOZLINA 1998 in culture of *Centaurea ragusina*. This fact could be explained in a view of poor soil conditions on natural habitats of all species investigated.

Elongated shoots (2-4 cm), excised from multiple shoot cultures were rooted on 1/2 MS medium containing 30 gl^{-1} sucrose and 8 gl^{-1} agar supplemented with different concentrations of IBA (2.5, 4.9 and 8.6 μM) or IAA (2.9, 5.7, 8.5 and 11.4 μM). The shoots of *Degenia velebitica* rooted only on medium containing 4.9 μM IBA but in a very low percentage (16.7%). That value is significantly lower in comparison with *Fibigia triquetra* shoot rooting (PEVALEK-KOZLINA & al. 1997). Adjusting of media to higher pH value (7.0), which is more similar to the soil pH value on natural habitat of species, did not affect rooting of *Degenia*

velebitica. In *Fibigia triquetra* cultures promotion of rooting through higher medium pH value was described (PEVALEK-KOZLINA & al. 1997), although lower pH values were suggested for related Brassicaceae species (GEORGE & SHERRINGTON 1984). The plantlets with well developed roots were transferred to the soil and successfully adapted to the outdoor conditions.

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