Botanica SERBICA 34 (2): 153-161 (2010)

Original Scientific Paper

In vitro propagation of *Dianthus ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* (Caryophyllaceae) from stem segment cultures

Ljiljana Radojević[1], Dušica Ćalić-Dragosavac[1], Jovanka Špirić[1], Branka Stevanović[2] and Vladimir Stevanović[2]

1 Institute for Biological Research ''Siniša Stanković", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia

2 Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

ABSTRACT: Plant regeneration of carnation species *Dianthus ciliatus* ssp. *dalmaticus* and *Dianthus giganteus* ssp. *croaticus* was achieved through micropropagation from apical and nodal segments culture on MS_2. Shoots multiplication was successful on the same medium *via* axillary buds. There were differences between multiplication index (MI) of shoots originating from apical and nodal basal stem segments. Nodal segment shoots had a better MI (*D. ciliatus* ssp. *dalmaticus* = 7.86; *D. giganteus* ssp. *croaticus* = 0.68) than apical ones (*D. ciliatus* ssp. *dalmaticus* = 6.94; *D. giganteus* ssp. *croaticus* = 0.50). Shoots of both species were rooted on MS_0 without hormones, MS_3 and MS_4. Adventitious buds (AB) and somatic embryo like structures (ES) were formed after the transfer of green-yellow callus from MS_5 to MS_6. Further development and multiplication of AB and ES were achieved on medium MS_7. Plants formation was brought about by micropropagation of shoots, organogenesis and/or somatic embryogenesis.

In vitro plantlets of both carnation species were planted in rocky garden of the Belgrade Botanical Garden ''Jevremovac" where they bloomed. Subsequently, these *in vitro* plantlets will be reintroduced in natural environment.

Key words: *Dianthus ciliatus* ssp. *dalmaticus*, *Dianthus giganteus* ssp. *croaticus*, adventitious buds, somatic embriogenesis, somatic embryo like structure, micropropagation, organogenesis, stem segment culture

Abbreviations: BAP- 6-benzylamino purine; 2,4-D-dichlorphenoxyacetic acid; Kin- 6-furfurylamino purine; NAA- alfa-napthaleneacetic acid; IBA- 2-indiole-butyric acid; GA_3- gibberellic acid; TDZ-thidiazuron; MS- Murashige and Skoog's (1962) mineral solution; AB- adventitous buds; AS-apical segment; NS-nodal segment; EC-embryogenic callus; ES-embryon like structure; OC-organogenic callus.

Received 12 November 2010 Revision accepted 24 December 2010

UDK 582.661.51.085.2

INTRODUCION

The genus *Dianthus* L. (fam. Caryophyllaceae) includes many perennial and a few annual or biennial herbaceous species and very seldom low subshrubs with woody basal stems. Nodes on the stem are swollen; leaves are simple, linear, opposite, grey to blue green in colour. The flowers are hermaphroditic, terminal, solitary or in dichasial cymes, pale to dark pink. Otherwise, the genus was named *Dianthus* by C. Linnaeus, coined by Greek words *dios* – God and *anthos* – flower, i.e. „divine flower". The genus *Dianthus* has about 300 species, native to Europe and Asia.

*correspondence: ljradojevic@sbb.rs

In the Balkan Peninsula there were recorded about 170 species of which almost 100 are endemics. They inhabit various types of geological substrata (sand, loess, lime, dolomite, silicate, serpentinite), in open habitats such as sands, steppes, hilly and mountainous meadows and stony grounds, from lowland regions to mountain tops over 2500 m. The carnation plants prefer neutral to slightly alkaline, well-drained soil. They grow mostly in full sun but also tolerate light shade.

Many species and more than 100 hybrids of the genus *Dianthus* are used as ornamental plants of rocky gardens, owing to its remarkably beautiful fragrant blossoms that appear during the spring and summer, and often deep into the autumn. Besides carnation plants are often used as cut-flowers, and given that flower colour of dry plants is retained they are useful for pressed flower crafts and arrangements. For flower industry/horticulture of particular significance are species having beautiful, large flowers, of different shades of pink to red, which are distinguished by long blossoming period, thriving on poor, acid soils and drought resistance (Spinski *et al.* 1974; Chouch & van Staden 1993; Radojević *et al.* 2010). Such species and hybrids are common in parks, especially in rocky gardens, as floral edges of seedbeds, or as covers of barren sites in settlements (Radojević 2007).

The endemic species are of particular interest, bearing in mind the need of protection and preservation of species and genetic diversity of these, more or less, threatened plants as well as their ornamental characteristics. In the region of the Balkan Peninsula to endemic carnations belong *Dianthus ciliatus* Guss. and *D. giganteus* Dum.-Urville.

D. ciliatus ssp. *dalmaticus* (Celak.) Hayek is an endemic species spread in the Mediterranean and Submediterranean zone of the Adriatic coast in Dalmatia (Croatia) and Montenegro (Fig.1a). It is low semi-woody shrub.

D. giganteus ssp. *croaticus* (Borbás) Tutin is a subendemic species distributed in the western part of the Balkan Peninsula, from Slovenia eastward to Serbia (Fig. 1b). It is a perennial herb forming loose cushions.

Hitherto only several papers concerned with conservative micropropagation of endemic species of carnation were published (Radojević *et al.* 1997; Berardi *et al.* 2004; Cristea *et al.* 2004; Jain *et al.* 2006; Sarasan *et al.* 2006).

The aim of the present study was to form a great number of *in vitro* plants by applying stem segment culture both for the purpose of preserving genopool of these species and for setting up an important resource for the horticultural application of endemic carnations as ornamental plants. According to available data, species *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* till now have not been introduced to *in vitro* culture.

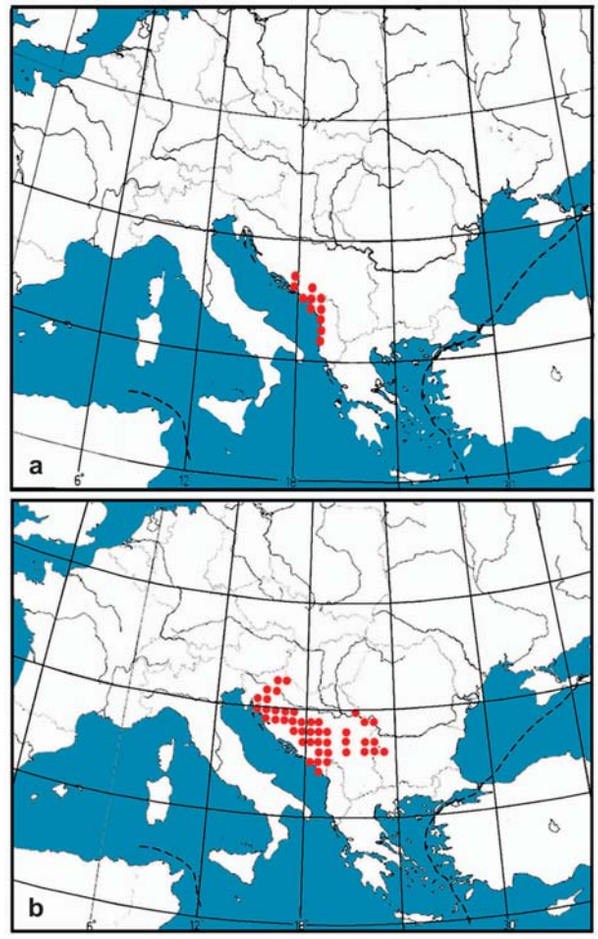

Fig. 1. Distribution of *D. ciliatus* ssp. *dalmaticus* (a); distribution of *D. giganteus* ssp. *croaticus* (b). (according to Jalas & Suominen, 1986)

MATERIAL AND METHODS

Seed germination and seedling formation. *In vitro* culture was initiated with seeds of *D. ciliatus* ssp. *dalmaticus* from Mt Lovcen (Montenegro) and *D. gigantheus* ssp. *croaticus* from Vlasina Lake (Serbia). The seeds were washed with plenty of running water (2 h) and then immersed in 20 % commercial bleach by modified procedure of Radojević *et al.* (1990) for 20 min, rinsed with sterile distilled water and cultivated in Petri dishes (10 seeds per dish) containing sterile filter paper with distilled water for germination under dark (10 days). Petri dishes were sealed with Parafilm (Pechiney Plastic Packing, Chicago, IL, USA). Non-contaminated germinated seeds (Plate I, Fig. 1), freed from seed coat were transferred in new Petri dishes containing the MS_1=MS_0+ GA_3 (1.0 mg L^{-1}) medium for seedlings formation and elongation (Plate I, Fig. 2).

Culture media. Initial apical and nodal segments (about 3-4 mm long) were cultivated on MS_2=MS_0+ NAA 1.0 +

IBA 0.5 + BAP 1.0 (mg L^{-1}, of each) for shoot induction and formation (Plate I, Figs. 3 and 9). These explants were placed in tube (10.0 x 1.0 cm) containing 8 ml of the MS_2 medium.

In the second subculture, only some explants formed shoots, about 1-2 cm (Plate I, Figs. 4 and 10). These shoots were subcultured on the same medium (40 ml/jar of 250 ml). Cultures were continuously grown on MS_2 medium (100 ml of medium/jar of 500 ml with aeration) for shoot multiplication.

Shoots of both species were rooted on MS_0 without hormones, MS_3 and MS_4 = MS_0+ IBA (0.5-1.0 mg L^{-1}, respectively) media.

Culture media for organogenesis and/or somatic embryogenesis induction. Sporadically, some of basal part of shoots, in contact with media, was formed being either compact green and/or loose white calli (Plate I, Fig. 3). Separated calli were subcultured on MS_5 =MS_0+ 2,4-D 5.0 + BAP 1.0+ NAA 1.0 (mg L^{-1}, each), and then transferred on MS_6 =MS_0+ 2,4-D 1.0 + BAP 1.0 + NAA 0.1 (mg L^{-1}, each) media for further differentiation.

Only calli with adventitious buds (AB) and/or embryo like structure (ES) were transferred on MS_7 =MS_0+ 2,4-D 1.0 + BAP 1.0+ NAA 5.0+ TDZ 0.2 (mg L^{-1}, each) medium. Duration of this subculture was 30 days.

All media (MS_1-MS_7) contained MS mineral solution (Murashige & Skoog 1962), supplemented with 2% sucrose, 0.7% (w/v) agar and (in mg L^{-1}): nicotinic acid 10, panthothenic acid 0.1, folic acid 0.01, ascorbic acid 10, vitamin B_1 2.0, riboflavin 0.2 and myo-inositol 100 (medium MS_0). The media (MS_1-MS_7) were gelled with 0.7% agar (w/v) and pH was adjusted to 5.8 with 1 N NaOH before sterilization. All media were sterilized by autoclaving at 0.9 x 10^5 kPa and 114^{0}C (25min). Cultures were grown at 25±1^{0}C, under 16 h photoperiod and 8 h dark.

Anatomical examination of the calli. All types of calli, i.e. embryogenic callus (EC) and organogenic callus (OC), were fixed in FAA (formalin: acetic acid: ethanol 5:5:90) during 24 h at 4°C. The tissues were dehydrated in an ethanol series and the permanent preparations were obtained by standard paraffin technique and hematoxiline and/or toluidine blue staining procedures (Martoja & Martoja 1967).

Statistics and repetition. The results were assessed using the variation analysis in Statistica 10.0. The means were compared by the least significant difference (LSD) test (significance level $p<0.05$). Three repetitions had been performed per each medium.

Table 1. Formation of seedlings of *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* in *in vitro* culture. Each data is mean of three replicates.
*values in each column marked by different letters are significantly different at 0.05 using the LSD test.

Species	Number of seeds in culture	Medium for seedling formation (hormone in mg L^{-1})	Number of seedlings	% of seeds germination
D. ciliatus ssp. *dalmaticus*	149	MS + 3 % sucrose + GA_3 1.0	105	70.46^{a*}
D. giganteus ssp. *croaticus*	158	MS + 3 % sucrose + GA_3 1.0	66	41.77^{b}

Table 2. Shoot multiplication index of apical (AS) and nodal (NS) segments in *in vitro* culture of *D. ciliathus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* seedlings.
*means ± SE (standard error); **values in each column marked by different letters are significantly different at 0.05 using the LSD test.

Species	Date	Medium	Total number of shoots for multiplication	The average length of shoots (in cm)	The average N^o roots/ plantlet
D. ciliatus ssp. *dalmaticus*	08.02.2007	MS_0	27	8.67 ± 0.35*	7.33 ± 1.58^{a**}
		MS_3	15	9.43 ± 0.67	5.06 ± 0.77^{ab}
	29.03.2007	MS_0	38	8.85 ± 0.76	7.52 ± 1.00^{a}
		MS_3	15	6.71 ± 0.88	6.40 ± 0.79^{ab}
D. giganteus ssp. *croaticus*	26.06.2006	MS_0	15	5.66 ± 0.89*	3.00 ± 0.43^{b}
		MS_3	30	8.22 ± 0.96	7.85 ± 0.91^{a}
	16.08.2006	MS_0	15	2.60 ± 0.35	0
		MS_3	30	6.06 ± 0.28	5.15 ± 0.76^{ab}

PLATE I. Figs. 1-15. Micropropagation in stem segment culture of *D. ciliatus* ssp. *dalmaticus* and *Dianthus giganteus* ssp. *croaticus*. Germinated seed (**1**) and seedling (**2**) of *D. ciliatus* ssp. *dalmaticus*; the first subculture and shoots multiplication of *D. ciliatus* ssp. *dalmaticus* (**3**) and of *D. giganteus* ssp. *croaticus* (**4**) on the medium MS_2; rooted shoot of *D. ciliatus* ssp. *dalmaticus* (**5**) on medium MS_3; *in vitro* plants of *D. ciliatus* ssp. *dalmaticus* in early (**6**) and later acclimatation in greenhaus (**7**); flowering *in vitro* plants of *D. ciliatus* grown in Botanical Garden ''Jevremovac'' (**8**), the first subculture (**9**) and shoots multiplication of *D. giganteus* ssp. *croaticus* on MS_2 medium (**10**); plants of the same species in early and later acclimatization stage (**11, 12, 13**); flowers (**14**) and mature seed capsules (**15**) of *D. giganteus* ssp. *croaticus* reintroduced in Belgrade Botanical Garden ''Jevremovac''

RESULTS AND DISCUSSION

Protocol was designed for establishing initial culture of shoots from apical segments (AS) and nodal segments (NS) of seedlings of the species *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus*. Seeds of these species, germinating on MS_1 medium with 2% saccharose and GA_3 1.0 mg L^{-1}, had different germination rate (Table 1, Plate I, Figs. 1 and 2).

In the initial phase of seed cultures of *D. ciliatus* ssp. *dalmaticus* there were 46.42 % of infected seeds, whereas for *D. giganteus* ssp. *croaticus* seed infection was somewhat lower (39.28%). Infection considerably affected seed germination given that it decreased a number of viable seeds. Percentage of seed germination of *D. ciliatus* ssp. *dalmaticus* was markedly higher (70.46%) in respect to seed germination of *D. giganteus* ssp. *croaticus* (41.77%). Species specificity also affected multiplication of shoots.

Apical segments (AS) and nodal segments (NS) of *D. ciliatus* (Plate I, Fig.3) and *D. giganteus* (Plate I, Fig. 9) explants have formed clusters of small shoots already after the first subculture on the medium MS_2.

Multiplication of segments occurred *via* axillary buds on the same medium after the first subculture. Shoot multiplication was monitored from the first to the fourth subculture (Table 2, Plate I, Figs. 4 and 10).

The positive gradient of multiplication index (MI) was recorded in the culture of AS and NS segments for both of *D. ciliatus* ssp. *dalmaticus* and of *D. giganteus* ssp. *croaticus* with the subsequent subcuturing (namely II, III and IV subculture). MI had the highest value in the subculture IV, being always significantly higher in *D. ciliatus* than in *D. giganteus* ssp. *croaticus* (Table 2). In both species nodal segments had a better MI than apical segments which is in agreement with the results of Cristea *et al.* (2004; 2006) for some other endemic species of the genus *Dianthus* from Romania or other regions of Europe (*D. alpinus* L., *D. ferrugineus* Miller, *D. gallicus* Pers., *D. giganteus* subsp. *banaticus* (Heuffel) Tutin, *D. gratianopolitanus* Vill. and *D. henteri* Heuffel ex Griseb. & Schenk). Specially, for *D. henteri* the nodes have a better multiplication rate than apexes segments (Cristea *et al.* 2010).

For micropropagation of *Dianthus* species the most often used of auxins are IAA and NAA, and of cytokinins, Kin or BAP (Stone 1963; Davis *et al.* 1977). Combination of two auxins (IBA and NAA), was used in the medium for the multiplication of shoots in twelve varieties of carnations important for horticulture (Radojević *et al.* 1990; 1994; Radojević 2007). In addition, both auxins (NAA and IBA) were used for the micropropagation of shoots of endemic species of carnations: *Dianthus petraeus* Waldst. & Kit. (Radojević *et al.* 1997; Jain *et al.* 2006), *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* (Radojević *et al.* 2006). Otherwise, for the multiplication of shoots of *D. ciliatus* ssp. *dalmaticus and D. giganteus* ssp. *croaticus,* only cytokinin BAP was used, which was in line with the results for *D. caryophyllus* L. (Woo & Park 1993; Gharbia *et al.* 2008), *D. petraeus* (Radojević *et al.* 1997), *D. superbus* L. (Mikulik 1999) *D. balbisii* Ser. (Berardi *et al.* 2004) and *D. elegance* Dum.-Urville (Baktir & Hazar 2004). In general, in certain carnation species cultures during multiplication phase vitrified shoots were observed, which resulted in malformation of some shoots that were excluded from further propagation. Occurrence of vitrification in shoots of carnations in the presence of BAP was first described by Roest & Bokelmann (1981). Otherwise, vitrification of carnation shoots is very common phenomenon during micropropagation. According to the results of Messeguer *et al.* (1993) and Radojević *et al.* (1994) BAP concentration higher than 1.0 mg L^{-1} increased a number of vitrified shoots. Higher ratio of NO_3-/NH_4+ in the medium prevented the vitrification of carnation plantlets (Tsay 1998). However, a suitable protocol to overcome hyperhydracity in carnation during micropropagation was developed by Yadav *et al.* (2003). In our experiments the use of optimal BAP concentration (1.0 mg L^{-1}) and growing of shoots in the aerated jars did not lead to vitrification during propagation in *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus.*

Carnation shoots were mostly rooted on media without hormones (Leshem 1986), or on those with auxins (Petru & Landa 1974; Radojević *et al.* 1997). The shoots of *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* were rooted on MS_0, MS_3 and MS_4 media. Shoot rooting of both carnation species depended on medium, shoot length and the season in which it occurred. According to the results the most suitable for shoot rooting were the shoots 8.85 cm and 8.22 cm long of *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus*, respectively (Table 3). The most favourable conditions for the rooting of *D. ciliatus* ssp. *dalmaticus* shoots was in March (7.52 roots/plantlet) on medium MS_0 without hormones, whereas the most favourable period of rooting for *D. giganteus* ssp. *croaticus* was June (7.85 roots/plantlet) on MS_3 medium (Table 3, Plate I, Fig.5).

After the first passage, a certain number of shoots in the culture (about 10 %) has formed (OC) organogenic greenish-yellow compact (Plate II, Figs. 1 and 2) and (EC) loose white calli (Plate II, Fig.3). These calli were grown for the purpose of further differentiation on the medium MS_5. After one month these calli were transferred onto the medium MS_6 to induce organogenesis. After the second subculture adventitious buds (AB) and a smaller number of proembryo like structures (ES) were formed (Plate II, Fig. 4).

Only calli that formed AB (Plate II, Figs. 1, 2 and 5)

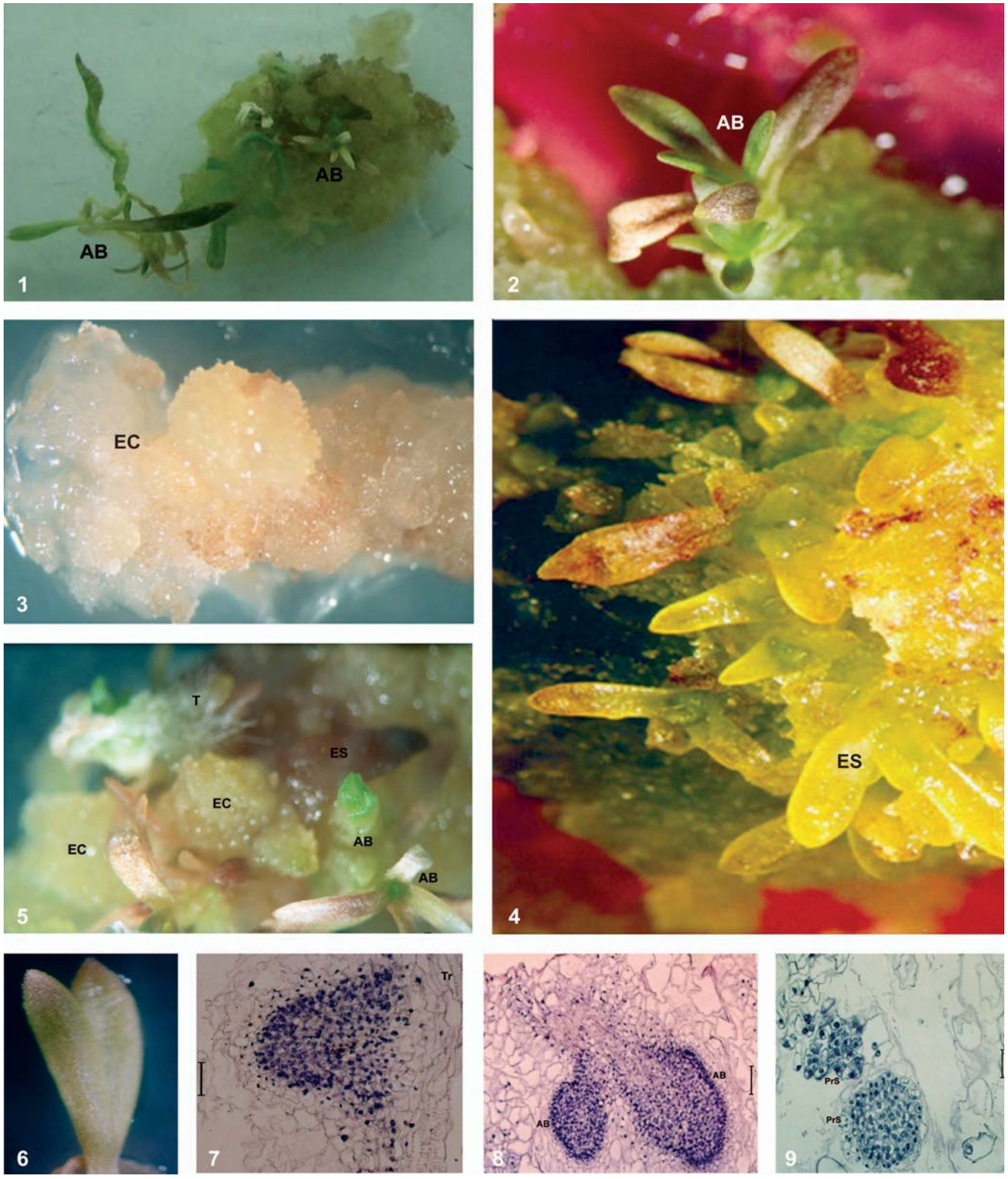

PLATE II. 1-9. Different morphogenic response in callus cultures of *D. ciliatus* ssp. *dalmaticus*. Organogenic callus with adventitious buds (AB) on the medium MS_5 (**1,2**); embryogenic callus (EC) (**3**); EC with embryos like structure (ES) (**4**); AB, EC, ES and trichomes (T) (**5**) on the medium MS_6; emblings of *D. ciliatus* ssp. *dalmaticus* with two cotyledons on the medium MS_7 (**6**); early stages of adventitious bud formation with tracheides (Tr) (**7**); latter stages of adventitious buds (AB) (**8**); Proembryo like structure (PrS) (**9**)

Remarks: 7-9. Fixation: FAA; 7 and 8. Staining: hematoxyline; 9. Staining: toluidine blue; 7-9. Bar=50μm.

Table 3. Shoots rooting of *Dianthus giganteus* subsp. *croaticus* and *Dianthus ciliatus* ssp. *dalmaticus*.

Species	Segments		Medium	Shoots multiplication index (MI)		
	Type	Number		Subculture		
				II	III	IV
D. ciliatus ssp. *dalmaticus*	AS	15	MS_2	0.58	5.35	6.94
	NS	126		1.37	7.41	7.86
D. giganteus ssp. *croaticus*	AS	18		0.16	0.35	0.50
	NS	34		0.32	0.50	0.68

and/or ES structures (Plate II, Fig. 6) were transferred on medium MS_7 for further development and multiplication. According to data from available literature, TDZ containing media are very efficient for the induction of somatic embryogenesis and for the multiplication of somatic embryos (Visser *et al.* 1992; Mithila *et al.* 2003; Panaia *et al.* 2004).

Anatomical examinations of OC confirmed that during the first subculture on MS_5 and after on MS_6 medium meristem zones appeared and were quickly formed into initial nodular bumps like shoots. During further development, vascular elements were differentiated within these structures (Plate II, Figs.7 and 8). Serial sections of regenerated structures did not reveal bipolarity, thus confirming that morphogenesis proceeded in the direction of organogenesis. In the present study, in the culture of stem segments of both *Dianthus* species, embryogenic calli were developed on the same transfer media (from MS_5 to MS_6). Reduction of 2,4-D in these media led to teratological callus differentiation in OC, EC and to formation of proembryo like structures at EC callus (Plate II, Figs. 6 and 9).

Micropropagation was possible *via* shoot culture deriving from different segments of seedlings. *In vitro* plantlets of *D. giganteus* ssp. *croaticus* had large ornamental flowers with long flower stalks of average length of 86.14 cm, while flowers of *D. ciliatus* were smaller with shorter flower stalks (48.10cm) Due to its ornamental characteristics and the duration of flowering period this species might be used in flower industry/horticulture, e.g. "cut flowers", whereas *in vitro* plants may be used for further research of hybridization (Wen Guabao *et al.* 1995). In the other hand, the micropropagated plantlets of *D. ciliatus* might be used in park horticulture as floral edges of seedbeds, or as covers of barren sites in settlements (Radojević 2007).

Preliminary anatomical studies concerned with callus of *D. ciliatus* indicate that simultaneous induction of organogenesis and somatic embryogenesis is possible as previously has been reported for the embryos culture of *Iris pumila* (Radojević *et al.* 1987) and *Iris setosa* (Radojević & Subotić 1992).

The regeneration protocol presented here for conservative micropropagation (Radojević *et al.* 2010) and plant regeneration of two endemic *Dianthus* species can be useful for the "large scale" multiplication of *in vitro* plantlets. These plants were used for the reintroduction in natural habitats. In addition, *in vitro* plantlets may serve as a complementary resource for transgenic plant production that might to be a subject of future studies.

CONCLUSIONS

Micropropagation of two *Dianthus* species was achieved by stem segments culture. Formation of adventitious buds was possible *via* organogenesis for endemic species, *D. ciliatus* ssp. *croaticus* and *D. giganteus* ssp. *croaticus*. In white callus of *D. ciliatus* ssp. *croaticus* the formation of embryogenic like structure is probably possible *via* somatic embryogenesis. Also, earlier preliminary cytological studies showed that these two processes (orgnogenesis and somatic embryogenesis) may occur simultaneously in the calli of *D. ciliatus* (Radojević *et al.* 2006, Radojević *et al.* 2010), but orgnogenesis was more pronounced than somatic embryogenesis. *In vitro* plantlets of both endemic carnation species were planted in rocky garden of the Belgrade Botanical Garden ''Jevremovac" where they even bloomed.

Acknowledgement – This study was financially supported by grants of the Ministery for Sciences and Technological Development of Serbia (No. 1573 and 143015).

REFERENCES

Baktir I & Hazar D. 2004. *In vitro* propagation of *Dianthus elegans*. 5th Symposium ''*In vitro* Culture and Horticulture Breeding" (IVCHB), 12-14 September, 2004, Debrecen, Hungary, p. 162.

Berardi G, Roncasaglia R, Scaravelli D & Dradi G. 2004. *In vitro* propagation of *Dianthus balbisii* Ser. ssp. *liburnicus* (Bartl.) Pign. by shoot tip culture. 5th IVCHB Symposium I, 12-14 September, 2004, Debrecen, Hungary, p. 164.

Chouch NR & van Staden J. 1993. *In vitro* culture of *Dianthus zeyheri* subsp. *natalensis*, a South African carnation. *Plant Cell, Tissue and Organ Culture* **35**: 81-85.

Cristea V, Brummer AT, Jarda L & Miclăuş M. 2010. *In vitro* initiation and phytohormonal influence on *Diathus henteri* - Romanian endemic species. *Romanian Biotechnological Letters* **15**(1): 25-33.

Cristea V, Miclăuş M, Puşcas M & Deliu C. 2004. Conservative micropropagation of some endemic or rare species from *Dianthus* Genus. 5th IVCHB Symposium I, 12-14 September, 2004, Hungary, p. 143.

Cristea V, Puscas M, Miclăuş M & Deliu C. 2006. Conservative micropropagation of some endemic or rare species from the *Dianthus* genus. *Acta Horticulturae* **725**: 357-364.

Davis MJ, Baker R & Hanan JJ. 1977. Clonal multiplication of carnation by micropropagation. *J. Am. Soc. Hort. Sci.* **102**: 48-53.

Gharbia HD, Atheel NY & Mobasher O. 2008. Clonal propagation of *Dianthus caryophyllus* L. through tissues culture. *J. Duhok Univ*. **12**(1): 91-95.

Jain SM, Jenks MA, Rout GR & Radojević Lj. 2006. Micropropagation of ornamental potted plants. *Propagation of Ornamental Plants* **6**(2): 67-82.

Jalas J., Suominen J. (eds.). 1986. Atlas Florae Europaeae 7, Caryophyllaceae (Silenoideae), The Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo, Helsinki.

Leshem B. 1986. Carnation plantlets from vitrified plants as a source of somaclonal variation. *Hort. Sci.* **21**: 320-321.

Martoja R & Martoja M. 1967. Initiation aux techniques de l'histologie animal. Masson et Çie, pp. 163-203.

Messeguer J, Arconada MC & Mele E. 1993. Adventitious shoot regeneration in carnation (*Dyanthus caryophyllus*). *Ann. Bot.* **68**: 563-568

Mikulik J. 1999. Propagation of endangered plant species by tissue cultures. *Acta Universitatis Palackianae Olmoucensis Facultas Rerum Naturalium, Biologica* **37**: 27-33.

Mithila J, Hall JC, Victor JM & Saxena PK. 2003. Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations of leaf and petiole explants of African violet (*Saintpaulia ionatha* Wendl.). *Plant Cell Report* **21**(5): 408-414.

Murashige T & Skoog F. 1962. A revised medim for rapid growth and bioassays with tabacco tissue cultures. *Physiol. Plant.* **15**:473-497.

Panaia M, Senaratna T, Dixon KW & Sivasithamparam A. 2004. The role of cytokinins and thidiazuron in the stimulation of somatic embriogenesis in key memebers of the Restionaceae. *Aus. J. Bot.* **55**: 257-265.

Petru E & Landa Z. 1974. Organogenesis in isolated caranation plant callus tissue cultivated *in vitro*. *Biol. Plant.* **16**: 450-453.

Radojević Lj. 2007. Primena *in vitro* kulture u proizvodnji cveća: Stanje i mogućnosti razvoja cvećarstva u Srbiji. Seminar pejzažne hortikulture, 8-9 februar, 2007, Šumarski Fakultet, Beograd, Zbornik predavanja, pp. 46-48.

Radojević Lj & Subotić A. 1992. Plant regeneration of *Iris setosa* Pall. through somatic embryogenesis and organogensis. *J. Plant.* **139**: 690-696.

Radojević Lj, Djorđević N & Petrović J. 1990. *In vitro* culture techniques for carnation breeding. *Acta Horticulturae* **180**: 163-168.

Radojević Lj, Marinković N & Jevremović S. 1997. Vegetativno raznožavanje u kulturi meristema i segmenata stabla *Dianthus petreus* Waldst et Kit. subsp. *noeanus*. *Glasnik Botaničke bašte Univerziteta u Beogradu* **31**: 1-7.

Radojević Lj, Sokić O, Tucić B. 1987. Somatic embryogenesis in tissue culture of *Iris* (*Iris pumila* L.). *Acta Horticulturae* **212**: 719-723.

Radojević Lj., Marinković N, Zdravković-Korać S & Jevremović S. 1994. Primena različitih postupaka *in vitro* kulture u mikropropagaciji afričke ljubičice, hrizantema i karanfila. *Savremena poljoprivreda* **42**(6):117-128.

Radojević Lj., Špirić J, Stevanović B & Stevanović V. 2006. *In vitro* culture of several endemic species *Dhianthus* in Balkan Peninsula. XXII Inter. Sym. Section Ornamentals 11-15 Sep. Sanremo, Italy, p 61.

Radojević Lj., Ćalić-Dragosavac D, Stevanović B. & Stevanović V. 2010. *In vitro* culture of several endemic species of genus *Dianthus* from Serbia and neighboring regions. 10th Symposium on the Flora of Southeastern Serbia and Neighbouring regions, Vlasina, 17-20 June, 2010, pp. 75-76.

Roest S & Bokelmann 1981. Vegetative propagation of carnation *in vitro* through multiple shoot development. *Sci. Hort.* **14**:357-366.

Sarasan VA, Crrips R, Ramsay R, Atherton MM, McMichen C, Prendergast M & Rowntree JK. 2006. Conservation *in vitro* of threatened plants-progress in the past decade. *In vitro Cell Development Biol. Plant.* **42**:20-25.

Spinski P. Beck GE & Mc Grown BH. 1974. Callus cultures of *Dianthus* species. *Hort Science* **9**:14.

Stone OM. 1963. Factors affecting the growth of carnation

plants from shoots apices. *Ann. Appl. Biol.* **52**:199-209.

Tsay HS. 1998. Effecat of medium composition at different recultures on vitrification of carnation (*Dianthus caryophyllus*) *in vitro* shoot proliferation. *Acta Hort.* **461**: 243- 249.

Visser C, Qureshi JA, Ravinder G & Saxena PK. 1992. Morphology role of thidiazuron: substitution of auxin and cytokinin requirements for the induction of somatic embryogenesis in geranium hypocotyl cultures. *Plant. Physiol.* **99**:1704-1707.

Wen G, Yu J, Zhao X, Fan G, Lin D & Yang Z. 1995. Studies on carnation (*Dianthus caryophillus*) species hybridization. *Acta Hort.* **404**: 82-90.

Woo S. H. & Park J. M. 1993. Multiple shoot culture *Dianthus caryophyllus* using mist culture system. *Bio. Tech.* **7**(10): 697-702.

Yadav M K, Gaur AK & Garag G K. 2003. Development of suitable protocol to overcome hyperhydricity in carnation during micropropagation. *Plant Cell Tissue Organ Culture* **72**: 153-156.

REZIME

In vitro umnožavanje u kulturi segmenata stabla of *Dianthus ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* (Caryophyllaceae)

Ljiljana Radojević, Dušica Ćalić-Dragosavac, Jovanka Špirić, Branka Stevanović, i Vladimir Stevanović

Regeneracija biljaka je postignuta u kulturi vršnih i nodalnih segmenata na podlozi MS_2. Umnožavanje izdanaka je bilo uspešno na istoj podlozi preko aksilarnih izdanaka. Multiplikacioni indeks (MI) izdanaka vršnih segmenta razlikovao se od MI izdanaka nodalnih segmenata. Izdanci poreklom od nodalnih segmenata imali su bolji MI (*D. ciliatus* ssp. *dalmaticus* = 7.86; *D. giganteus* ssp. *croaticus* = 0.68) od apikalnih segmenata (*D. ciliatus* ssp. *dalmaticus* = 6.94; *D. giganteus* ssp. *croaticus* = 0.50). Kod obe vrste, izdanci su bili ožiljavani na MS_0 podlozi bez hormona, i sa hormonima na MS_3 i MS_4. Adventivni pupoljci (AB) i strukture slične embrionu (ES) su bili formirani posle prenošenja žuto-zelenog kalusa sa podloge MS_5 na podlogu MS_6. Dalje razviće i umnožavanje (AB) i (ES) odvijalo se na podlozi MS_7. Formiranje biljaka je postignuto mikroprpagacijom izdanaka, organogenezom i/ili somatskom embriogenezom. *In vitro* biljčice karanfila obe vrste su bile prenete u alpinetum Botaničke bašte ''Jevremovac" u Beogradu gde su cvetale. U budućnosti, ove biljke mogu se reintrodukovati u prirodno stanište.

Ključne reči: *Dianthus ciliatus* ssp. *dalmaticus*, *Dianthus giganteus* ssp. *croaticus*, adventivni pupoljci, strukture slične embrionu, organogeneza, kultura segmenata stabla.