EX SITU CONSERVATION USING *IN VITRO* METHODS IN SOME *CARYOPHYLLACEAE* PLANT SPECIES FROM THE RED LIST OF VASCULAR PLANTS IN ROMANIA

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The diminishing of the genetic resources of the plants strongly imposed the development of new conservation techniques. *In vitro* techniques allow the increase of the propagation rate and the obtaining of a healthy material. In the case of the endangered plant species with a reduced number of individuals, seed sterility and low intrapopulational variability, the *in vitro* techniques represent the only viable conservation method.

The aim of our study is to elaborate micropropagation and conservation protocols for a medium-term period in some *Caryophyllaceae* endemic species.

The species taken in study are: *Dianthus callizonus* Schott et Kotschy, *Dianthus tenuifolius* Schur, *Cerastium transsilvanicum* Schur, *Dianthus spiculifolius* Schur. and *Dianthus superbus* L. ssp. *alpestris* Kablik ex Čelak.

Key words: in vitro, conservation, endemic Caryophyllaceae.

INTRODUCTION

The drastical reduction of the number of plant species from the entire world became the most important concern of botanists and ecologists.

While not all rare plants are endangered, the most endangered plants are almost always rare. The factors that determine the rarity are mainly the anthropic activities and randomic environmental events (the climate changes, the loss of seeds, and the failure of the pollination).

From about 12.000 plant species in Europe, over 2000 have been considered rare or endangered (3). In Romania, Boşcaiu *et al.* (3) specified 608 taxons with different degrees of vulnerability belonging to different IUCN categories (17.6% from the total number of species).

In the Red List of the Vascular Plants, Oltean *et al.* (17), mentioned 1438 species. From the total number of plants species indicated in this work, 74% are rare, 12% vulnerable, 3% endangered and 5 % extinct. Dihoru & Dihoru (8) mentioned that 4.5% species (1 189) from 3 976 species and subspecies belonging to Romania Flora are endemic.

Taking into account the diminishing of the genetic resources of plants, it is strongly imposed the development of new conservation techniques.

The genetic resources conservation is approached in two ways: by *in situ* conservation that involves the preservation and the management of the specific ecosystems and by *ex situ* conservation that involves the collections in the botanical gardens, the field cultures, the seeds storage and gene banks with the preserving *in vitro* plant tissue collections during different time intervals. In the whole world, over 10.000 threatened species are maintained in living collections (in botanical gardens, seed banks and tissue collections) representing about 30% of all known threatened species, a special attention being paid to the species with recalcitrant seeds.

The Convention on the Biological Diversity (1992) stresses the need of *ex situ* conservation made outside of the origin habitats using different methods (seeds banks, plants collections in field or in botanical gardens, tissues banks). The Global Strategy for Plant Conservation issued by The Convention on Biological Diversity has the main objective to stop the disappearance of endangered species and the protection of the existing ones.

Ex situ plant conservation using *in vitro* methods represents an alternative method for the protection and maintaining of the genetic fund of the endangered species (21, 11, 12, 22, 1, 2, 9, 10). *In vitro* cultures allow the increase of the propagation rate and the obtaining of a healthy material. In the case of the endangered plant species with a reduced number of individuals and a low intrapopulational variability, the *in vitro* techniques represent the only viable conservation method. The conservation using *in vitro* methods relies on the control of the growth of the inocula or of the cultured tissues (the halt of the growth or the reducing of it) (5).

The aim of our study is to elaborate micropropagation and medium term conservation protocols in some *Caryophyllaceae* endemic species.

The species taken into study are: ***Dianthus callizonus*** Schott et Kotschy, *Dianthus tenuifolius* Schur, *Cerastium transsilvanicum* Schur, *Dianthus spiculifolius* Schur. and *Dianthus superbus* L. ssp. *alpestris* Kablik ex Čelak.

All these species are mentioned in the Red List of Vascular Plants (16).

In Romania, there were made some studies concerning the possibility of conservation using *in vitro* methods in *Dianthus* genus species: *Dianthus spiculifolius* Schur (21; 4; 6), *Dianthus glacialis* Haenke ssp. *gelidus* (Sch., Nym et Ky.) Tutin – an endemic alpine species from Southern and Eastern parts of the Carpathians; *Dianthus spiculifolius* Schur – an endemic species of the Carpathians; *Dianthus petraeus W. et K. ssp. simonkaianus* (Péterfi) Tutin – found only in the Carpathians and the Balkan Mountains; *Dianthus giganteus* D'Urv – an important Balkan species; *Dianthus giganteus* D'Urv. ssp. *banaticus* (Heuff.) Tutin – an endemic species for Romania and Southern Serbia (7). Şuteu and Mocan (18) made some studies concerning the micropropagation in another relative endemic species – *Saponaria bellidifolia*.

MATERIAL AND METHODS

Different types of explants were used for the initiation of the *in vitro* cultures (Table 1). The best results were obtained in the case of the single node stem fragments and immature flower buds.

Table 1

The types of explants used for the initiation of *in vitro* cultures in five *Caryophyllaceae* species

No.	The species studied	The type of explant
1	*Dianthus callizonus* Schott et Kotschy	Foliar cuttings, single node stem fragments
2	*Dianthus tenuifolius* Schur.	Single node stem fragments
3	*Cerastium transsilvanicum* Schur.	Single node stem fragments
4	*Dianthus spiculifolius* Schur.	Flower buds, single node stem fragments
5	*Dianthus superbus* L. ssp. *alpestris* Kablik ex Čelak.	Flower buds, single node stem fragments

For the obtaining of the sterile explants, different plant organs were washed in running tap water for 2 hours, then dipped in 70° alcohol for 30 seconds and finally sterilised in $HgCl_2$ for 5–6 minutes, followed by three washings with sterile distilled water.

For the tissues cultures initiation and plants regeneration were tested many culture media variants having macro- and microelements according to the Murashige and Skoog formula (16), supplemented with B_5 vitamins (13).

The *in vitro* tissues cultures were tested for the regeneration capacity on different media variants, for establishing the most appropriate micropropagation and preserving protocols for each species studied. Different growth factors and supplements have been used. As carbon source sucrose was used and as nitrogen source glutamine, casein hydrolysate or yeast extract were used.

For the retention of the phenolic compounds released by plant tissues in the culture medium, active charcoal was added, and as antioxidant factor ascorbic acid was used.

For the induction of different *in vitro* developmental processes and for the maintenance of the tissue cultures, different media variants were used (Table 2).

The tissues cultures were maintained in the growth chamber, at 25 °C, at 4000 lux illumination and a photoperiod of 16/8 hours.

Table 2

The culture media variants tested for *Caryophyllaceae* endemic species

Components		Variants																	
		For induction and regeneration													For medium-term maintaining				
		M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M 18
Macro-nutrients		MS	MS	MS	MS 1/2	MS 1/2	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS 1/2
Micro-nutrients		MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
B Vitamins		B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B5	B 5
C vitamin (mg/l)		–	30	–	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Growth factors (mg/l)	BAP	1	1	1	1	0.1	1	2	2	1	–	–	1	1	–	–	–	–	–
	Kin	–	–	–	–	–	–	2	2	1	0.5	1.25	–	–	–	–	–	–	–
	ANA	0.1	0.1	0.1	0.1	0.01	0.1	0.2	–	0.25	–		0.1	0.25	–	–	–	–	–
	AIA	–	–	–	–	–	–	–	–	–	–	0.25	–	–	–	–	–	–	–
	2,4- D	–	–	–	–	–	–	–	0.2	–	1	–	–	–	–	–	–	–	–
	GA_3	–	–	–	–	–	–	–	–	0.5	–	–	–	–	–	–	–	–	–
	ABA	–	–	–	–	–	–	–	–	–	–	–	–	–	5	10	–	–	–
Carbon source (g/l)	Sucrose	30	30	30	30	20	30	30	30	30	30	20	30	30	20	20	20	20	10
Other supple-ments (g/l)	YE	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–
	CH	–	–	–	–	–	–	–	–	–	2	–	2	–	–	–	–	–	–
	Glut	–	–	–	–	0.25	–	–	–	–	–	0.6	–	–	–	–	–	–	–
	AC	–	–	0.5	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Man	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30	60	–
Agar (g/l)		8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
pH		5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8

Legend: MS – medium Murashige & Skoog (1962), B5 - Gamborg vitamins (1968); BAP – 6-benzyl aminopurine; Kin – kinetin; Zea – zeatin; ANA – alpha-naphthyl acetic-acid; AIA – beta indolyl acetic acid; IBA – indole 3 butyric acid; 2.4-D – 2,4 dichloro phenoxyacetic acid; GA_3 – gibberelic acid; ABA – abscisic acid; YE – yeast extract; CH – casein hydrolysed; AC – active charcoal; Glut – glutamine; Man – mannitol.

RESULTS AND DISCUSSIONS

All endemic *Caryophyllaceae* species tested showed *in vitro* response in our experimental conditions, but their reactivity was different. In all the cases, it could be induced and maintained successfully *in vitro* tissues cultures.

Dianthus callizonus was proved to be highly responsive to *in vitro* culture, but the reaction depends on the medium variant used. The explants represented by floral buds and single node stem fragments were suitable for the initiation of the tissues cultures.

Generally, the media variants with cytokinin dominance were favourable to the organogenesis process, allowing the multiple axillary shoots formation (Table 3).

The culture of the single node stem fragment on M1 medium variant determined the formation of axillary shoots with low rate (2–4 shoots/node).

The supplementation of the culture media with yeast extract, caşein hydrolysate, glutamine as nitrogen source, sustained the increase of the regeneration response (5–10 shoots/explant). Adding ascorbic acid as antioxidant factor in M2 variant and active charcoal in M3 variant and their combined using allowed the improvement of the multiplication rate, reaching 30 shoots/explant. The newly formed shoots showed an increased growth rate, being necessary their separation and culture on rooting medium. The reduction of the concentration of macro-nutrients from the Murashige-Skoog formula is also favorable to regeneration in this species.

The use of the regeneration media variants added with two types of cytokinins (benzyl-aminopurine and kinetin) associated with a low level of an auxin (naphthyl acetic acid or dichlorophenoxy acetic acid have determined an increased reactivity of the tissue cultured, the regeneration rate was over 50 shoots/ cultured explant. The M7 and M8 variants favoured the direct and indirect organogenesis process, the tissue cultures being very proliferative (Fig. 1).

Cultured on M9 variant, these proliferative tissues could be maintained over two months the transfer being not necessary (Fig. 2). In this species, no matter which regeneration variant was used, the rooting of shoots was easily done (Fig. 3). A lower rooting rate was registered on the medium supplemented with yeast extract.

The presence of dichlorophenoxy acetic acid combined with kinetin in M10 variant has favoured the induction of friable, non-regenerative calli.

Taking into account the high multiplication rate and the increased growth rate of the shoots formed in this species, it was strongly imposed the establishment of protocols of growth slowing for maintenance during medium term of tissues cultures.

Table 3

In vitro culture response of *Dianthus callizonus*

Medium variant	*In vitro* reactivity						
	Induction	**Callusing**	**Rhizogenesis**	**Multiple axillary shooting**	**Morphogenesis**	**No. regenerants/ explant**	**Medium-term maintenance**
M1	+	–	+	+	–	2–4 shoots	–
M2	++	–	+	++	–	5–15 shoots	–
M3	+	–	+	+	–	5–10 shoots	–
M4	+++	–	++	+++	–	20–30 shoots	–
M5	+++	–	++	+++	–	30 shoots	–
M6	++	–	–	++	–	5–10 shoots	–
M7	+++	+ –	+	–	+++	20–50 shoots	+
M8	+++	+ –	+	–	+++	20–50 shoots	
M9	++	–	+	–	+++	50 shoots	
M10	+++	+++		–	–	–	–
M11	++	++		–	–	–	–
M12	–	–	–	–	–	–	–
M13	+ –	–	+	+ –	–	1–2 shoots	–
M14	+	–	+	+ –	–	–	+ –
M15	+	–	+	+ –	–	–	–
M 16	+++	–	+	–	+++	20–30 shoots	+++
M 17	++	–	+	–	+++	10–20 shoots	++
M18	+	–	–	–	–	–	+ –

Legend: – negative *in vitro* response; + positive response; + – moderate response, ++ good response; +++ very good response.

The short and medium term conservation of the plant germoplasm involves the reduction of the growth rate (through diminishing of the mineral nutrients content, of the carbon source, the use of a medium stress factor, reduction of the temperature, of the light). This step is also necessary in other conservation procedures. The successful implementation of the minimal growth technologies involves the elaboration of specific protocols for every taxon taken into study. The simplest procedure is represented by the culture without transferring on the same medium during a long period of time. This is possible in the case of species with reduced *in vitro* reactivity and low growth rate. In *D. callizonus* cultures, the regenerated shoots had a good elongation rate, consuming the nutritive resources of the culture medium and finally they became etiolated.

For this reason, there were tested different variants with a reduced nutrients content (macro-nutrients and carbon source) and the use of agents which slow down the growth owing to the osmotic stress by supplementing the culture medium with mannitol. This treatment is based on reports that the slowing of *in vitro* cultures growth can be achieved by changing the osmotic potential of the cells in culture with high levels of osmolytes (23). Another method is the addition of growth retardants (14) such as abscisic acid, known to reduce the intracellular level of the water and enhance solutes accumulation (19).

The use of the variant of medium M8 with macro-elements of the Murashige-Skoog formula reduced half strengthen and sucrose reduced at 10 g/l did not permit the maintenance of the tissue cultures for more than two months, because the shoots continued to elongate.

The addition of abscisic acid in the culture medium helped to reduce the growth rate and sustain the organogenesis process, but the preservation of the cultures on this variant could not exceed three months; the shoots were etiolated.

The media variants supplemented with osmolyte mannitol in a concentration of 0.15 and 0.30 M allowed the optimal maintaining of the tissues cultures over six months with the strong stimulation of shoots formation through direct organogenesis (Fig. 4). These shoots did not grow; they remained at single node stage (Fig. 5). This plant material *in vitro* cultured can any time be transferred on appropriate medium to grow and to form developed plants. This protocol is adequate for the preservation of *in vitro* tissues cultures collection in a very reduced space and without any supplementary handling.

In ***Dianthus tenuifolius***, the single node stem explants were also suitable for *in vitro* culture initiation. Generally, the media with cytokinin dominance allowed the multiple axillary shoots formation (Table 4). Initially, on M1 medium variant, the formation of 2–3 shoots was induced, subsequently, after the culture on the variant supplemented with BAP/ANA 10/1 ratio, and ascorbic acid and/or active charcoal, the regeneration rate reached 20–40 shoots/explant (Fig. 6).

Table 4

Dianthus tenuifolius in vitro culture response

Medium variant	*In vitro* reactivity						
	Induction	Callusing	Rhizogenesis	Multiple axillary shooting	Morphogenesis	No. regenerants/ explant	Medium-term maintenance
M1	+ –	–	–	+ –	–	2–3 shoots	–
M2	+	–	–	+	–	8–12 shoots	–
M3	+	–	+	++	–	~ 20 shoots	–
M4	+++	–	+	+++	–	20–40 shoots	–
M5	+	–	+ –	+	–	2–4 shoots	–
M6	–	–	–	–	–	–	–
M7	+++	–	+	–	+++	20–50 shoots	
M8	+++	+ –	+	–	+++	20–50 shoots	
M9	+++	–	+	–	+++	50–100 shoots	
M10	–	–	–	–	–	–	–
M11	–	–	–	–	–	–	–
M12	–	–	–	–	–	–	–
M13	–	–	–	–	–	–	–
M14	–	–	–	–	–	–	–
M15	–	–	–	–	–	–	–
M 16	++	+	+	–	++	~ 20–30 shoots	++
M 17	+ –	+ –	+ –	–	+	10–20 shoots	+
M 18	+	–	+	+	–		+ –

Legend: – negative *in vitro* response; + positive response; + – moderate response ++ good response; +++ very good response.

The use of the combination of two cytokinins (BAP and kinetin) associated to an auxin (2,4-D or ANA) in M7 and M8 media variants allowed a higher multiplication rate (over 50 shoots/explant).

The culture of single node or two nodes explants on M9 variant (added with gibberelic acid) favoured the induction of direct neo-formed shoots with the highest rate (over 100/explant) (Fig. 7). The regenerated plants rooted both on hormone-free and on hormone-supplemented media (Fig. 8).

Concerning *in vitro* medium term conservation of this species, the behavior was similar to that of D. *callizonus*, the maintenance of the regenerative tissue cultures was performed on the media variants added with mannitol.

In ***Cerastium transsilvanicum***, although many types of explants were tested for *in vitro* culture initiation, only single node stem cuttings were suitable for this purpose. For instance, this species was weak responsive to the media tested.

The M10 variant allowed the formation of friable yellowish calli, while on M1 variant 2–3 lateral shoots developed, which easily rooted after their detachment.

The M4 variant with macro-nutrients MS reduced on half and supplemented with hormones such as BAP, NAA in 10/1 ratio and with ascorbic acid and active charcoal determined a regenerative response of 5–10 shoots/nodes. The use of a similar medium, but supplemented with the same growth factors diluted ten times and 20 g/l sucrose and 250 mg/l glutamine permitted the improvement of the regenerative response of 15–20 shoots/explant (Fig. 9). The medium term preservation of *in vitro* micropropagated shoots was performed through the culture on media supplemented with mannitol, which stimulated the organogenesis process (the induction of shoots).

***Dianthus spiculifolius* Schur.** and ***Dianthus superbus* L. ssp. alpestris (Kablik ex Čelak.)** showed a positive *in vitro* response in the case of flower buds explants and single node stem fragments. The behavior of the cultured explants on the tested media variants in *D. spiculifolius* and *D. superbus* species was similar (Table 5).

The culture performed on M1-M6 variants supplemented with BAP and NAA in 10/1 ratio also induced the multiple axillary shoots formation (20–30 shoots/nodal explant).

The rhizogenesis process occurred in all media tested, not being necessary a particular rooting medium (Figs. 10, 11). Our results are according to those reported by V. Cristea *et al.* (6) in a previous study. The subculture of the neo-formed shoots on M7, M8 and M9 variants determined the significant increase of the *in vitro* reactivity, of the number of the regenerants emerged through an indirect organogenesis process (50–100 shoots/explant). Mikulik (15) performed some studies concerning micropropagation in *D. superbus*, the best results of axillary shoot induction were obtained on medium supplemented with BAP, but the regeneration rate was lower than that we have obtained.

Table 5

Cerastium transsilvanicum in vitro culture response

Medium variant	*In vitro* reactivity						
	Induction	Callusing	Rhizogenesis	Multiple axillary shooting	Morphogenesis	No. regenerants/ explant	Medium-term maintenance
M1	–	–	–	–	–	–	–
M2	+	–	+	+	–	2–5 shoots	–
M3	+	–	++	+	–	3–5 shoots	++
M4	++	–	++	++	–	5–10 shoots	+
M5	+++	–	–	+++	–	15–20 shoots	++
M6	–	–	–	–	–	–	–
M7	–	–	–	–	–	–	–
M8	–	–	–	–	–	–	–
M9	–	–	–	–	–	–	–
M10	–	–	–	–	–	–	–
M11	–	–	–	–	–	–	–
M12	+	–	+++	+	–	2–3 shoots	–
M13	–	–	–	–	–	–	–
M14	–	–	–	–	–	–	–
M15	–	–	–	–	–	–	–
M 16	+	–	+	+	–	20–30 shoots	+
M 17	+ –	–	+ –	+ –	–	10–20 shoots	+ –
M 18	+	–	+	+	–	10–15 shoots	–

Legend: – negative *in vitro* response; + positive response; + – moderate response ++ good response; +++ very good response.

Table 6

Dianthus spiculifolius and *Dianthus superbus in vitro* response

Medium variant	*In vitro* reactivity						
	Induction	Callusing	Rhizogenesis	Multiple axillary shooting	Morphogenesis	No.regenerants /explant	Medium-term maintenance
M1	++	–	+	+	–	5–10 shoots	–
M2	++	–	+	++	–	10–15 shoots	–
M3	+	–	+	+	–	5–10 shoots	–
M4	+++	–	++	+++	–	20–40 shoots	–
M5	+++	–	++	+++	–	30 shoots	–
M6	++	–	–	++	–	5–10 shoots	–
M7	+++	–	+	–	+++	30–50 shoots	+
M8	+++	–	+	–	+++	30–50 shoots	
M9	++	–	+	–	+++	50–100 shoots	
M10	+++	–	–	–	–	–	–
M11	++	–	–	–	–	–	–
M12	–	–	–	–	–	–	–
M13	+ –	–	+	+ –	–	3–5 shoots	–
M14	+	–	+	+ –	–	–	+ –
M15	+	–	+	+ –	–	–	–
M 16	+++	–	+	–	+++	20–30 shoots	+++
M 17	++	–	+	–	+++	20 shoots	++
M18	+	–	+	–	–	–	+ –

Legend: – negative *in vitro* response; + positive response; + – moderate response, ++ good response; +++ very good response.

The growth rate was very high, for that reason it was strongly imposed the necessity of the culture on media for growth slowing down added with mannitol (0.15 and 0.30 M). The subcultures were performed once at 3–4 months. The developed plants can be any time *ex vitro* cultured (Fig. 12).

CONCLUSIONS

- ***Dianthus callizonus*** species is very reactive *in vitro* culture. The preliminary response of 2–4 shoots/node could be optimized, reaching 30–50 shoots/explant. The regenerated shoots formed roots easily, not being necessary a special culture medium. The efficient preservation of the regenerative cultures was made on medium supplemented with 0.15 mannitol, the proliferation rate being very good.
- *D. tenuifolius* had also a good *in vitro* reaction, but its response was initially lower compared to *D. callizonus*, subsequently, it could be obtained *in vitro* high responsive cultures. The *in vitro* medium-term maintenance was also efficiently made on mannitol-supplemented medium.
- ***Cerastium transsilvanicum*** showed *in vitro* culture a similar behavior to those of related species, but the multiplication rate was lower. The suitable regeneration way is also the axillary buds proliferation.
- In *Dianthus spiculifolius* and *Dianthus superbus species*, the *in vitro* response was alike.

  The culture of flower buds and single node stem fragments on media supplemented with BAP and ANA in 1/10 ratio determined the multiple axillary shoots formation with a range varying between 5–30 shoots/explant. The subsequent subculture of the neo-formed shoots on M7, M 8, M9 variants conducted to a significant growth of the *in vitro* reactivity, the number of shoots developed through indirect morphogenesis reaching 50–100/explant.
- Generally, in all *Caryophyllaceae* species tested, the growth rate is high, for *in vitro* maintenance being strongly imposed the use of a specific medium for growth slowing down supplemented with mannitol.
- The micropropagation and medium-term preservation protocols elaborated in our work can be used for the achievement of *in vitro* collection in these species. The regenerated plants could be also used for the re-introduction in the natural habitats.

Fig. 1. High rate organogenesis in *Dianthus callizonus*.

Fig. 2. *D. callizonus* direct organogenesis during cultivation on a M 9 medium.

Fig. 3. Regenerated shoots of *D. callizonus* cultured on hormone-free medium.

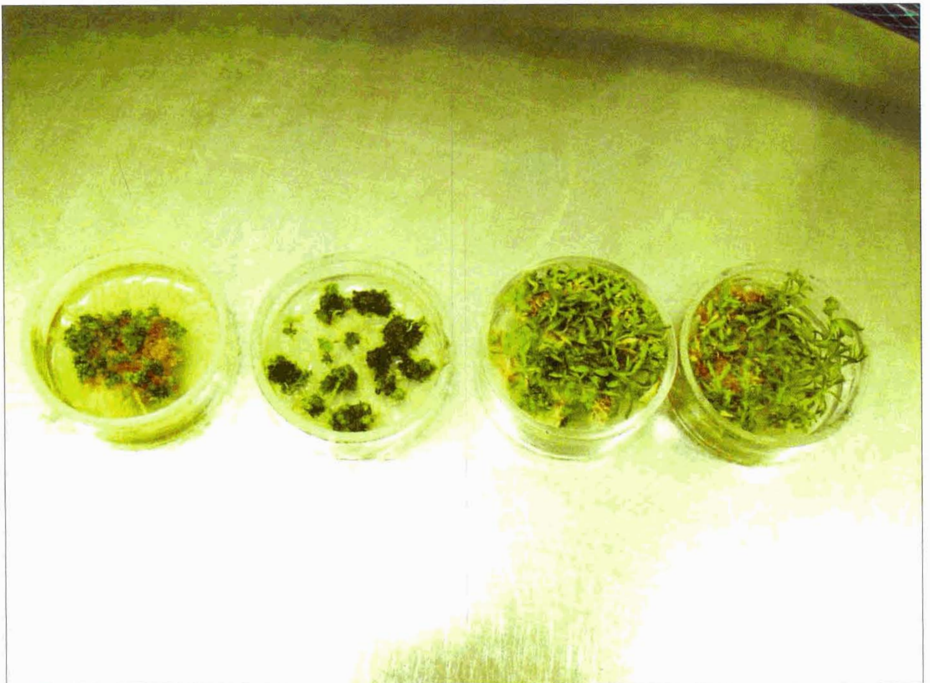

Fig. 4. Medium-term *D. callizonus* regenerative cultures maintained on different media (M17, M16, M 14, M 18).

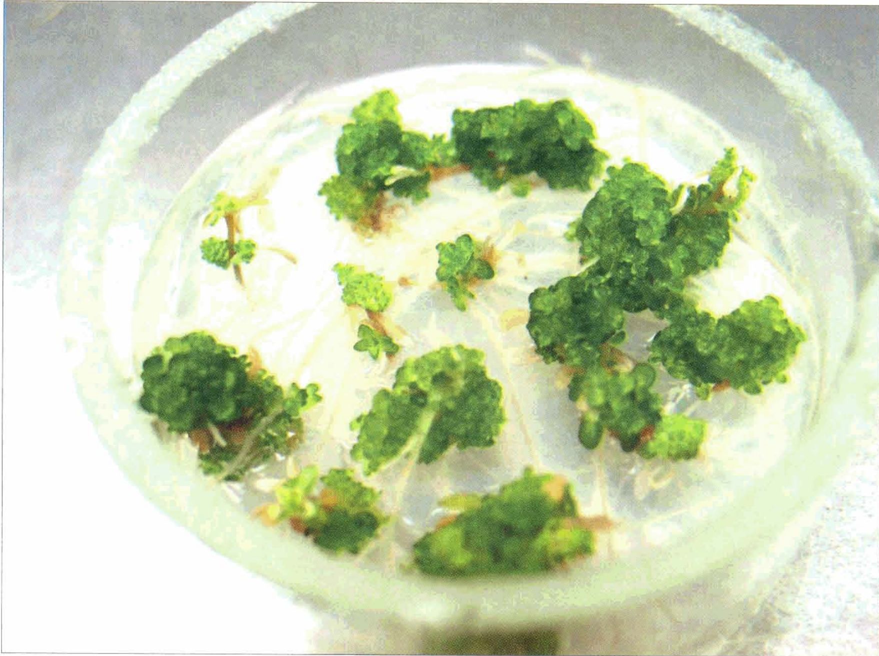

Fig. 5. *D. callizonus* single node shoots induced through direct morphogenesis on mannitol-supplemented medium.

Fig. 6. Multiple shoots developed on charcoal-supplemented regeneration medium M4 in *Dianthus tenuifolius*.

Fig. 7. Direct morphogenesis in *D. tenuifolius* on M9 regeneration medium.

Fig. 8. Regenerated rooted plants of *D. tenuifolius* cultured on hormone-free medium.

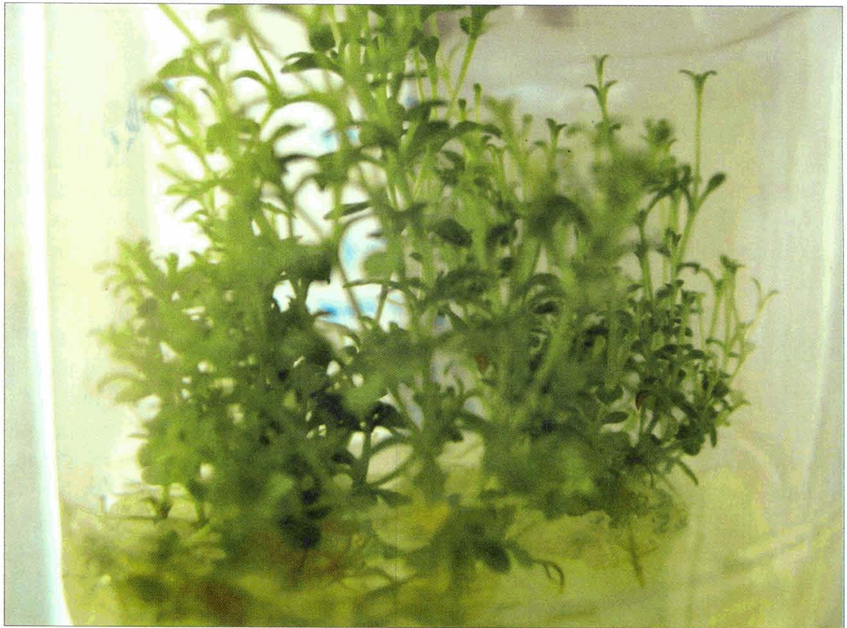

Fig. 9. Multiple axillary shooting in *Cerastium transsilvanicum*.

Fig. 10. *Dianthus superbus* regenerated plants through multiple axillary shooting.

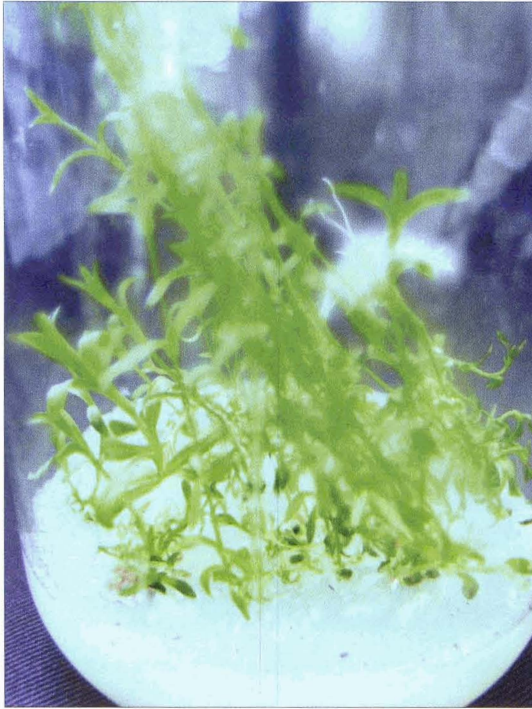

Fig. 11. *In vitro* regenerated plants of *Dianthus spiculifolius*.

Fig. 12. *Ex vitro D. spiculifolius* plant cultured in pot.

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