Direct somatic embryogenesis in dependent on the topophysical position of the explant in cactus *Copiapoa tenuissima* Ritt. forma *monstruosa*

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Abstract

Cactus *Copiapoa tenuissima* Ritt. f. *monstruosa* is a *C. tenuissima* Ritt. spontaneous mutant. This form has nearly black epidermis and it has no thorns in areoles, and it is very rare and attractive for collectors. Micropropagation is an attractive method for conservation and propagation of rare species of plants. Somatic embryogenesis is most efficient from all methods of multiplication. There was investigated the induction of direct somatic embryogenesis of *C. tenuissima* Ritt. f. *monstruosa* depending on the topophysical explant position on the donor plants. The explants were cultured on the modified medium with 2 mg·dm^{-3} auxin 2.4-D (2.4-dichlorophenoxyacetic acid) or MS medium without growth regulators (as control). The cultures were kept in a growth room at 24 ± 2°C and exposed to 16 h photoperiod. Daylight was by maintained using Philips TLD54/36 W lamps with the photon flux density of 38.1 μmol·m^{-2}·s^{-1}. The induction of somatic embryogenesis in the cacti *C. tenuissima* Ritt f. *monstruosa* was obtained only when the media were supplemented with auxin 2.4-D. However, most explants regenerated somatic embryos derived from the distal and central zone of main shoots of donor plants and from young axillary shoots (to 0.26 per one inoculated explant); yet from the proximal part of the main shoot of cacti, the number of explants which regenerated somatic embryos was low (0.02 per inoculated explant) and did not differ from the control.

Keywords: *Cacti, somatic embryo, topophysis*.

Introduction

Copiapoa tenuissima Ritt. (synonym *C. humilis* var. *tenuissima* Ritt.) comes from extremely dry desert areas of Chile; its epidermis is dark in color and it has wooly areoles (Graham, 1998). The forma *monstruosa* (synonym *Neochilenia wageringeliana* and *C. wageringeliana*) is a spontaneous mutant with its epidermis definitely darker in color (almost black) and no thorns in wool-like areoles (Dornig, 1976). These characteristics are highly valued by breeders and collectors of cacti, and they can be preserved by vegetative propagation. To propagate that valuable forma, micropropagation with the use of meristems and axillary shoot growth is applicable (Lema-Rumińska and Licznerska, 2004). However, even greater hopes for obtaining a large number of offspring plants are offered by somatic embryogenesis which can be applied both in conservation breeding to mass propagation, but also in creative breeding at the stage of plant regeneration and conservation of plants species (Moebius-Goldammer *et al*., 2003). The first study on somatic embryogenesis in cacti was made in *Neomammillaria prolifera* (Mill.) Britton & Rose (Minocha and Mehra, 1974). However, so far there are few applicable reports in cacti (Infante, 1992; Santacruz-Ruvalcaba *et al*., 1998; Da Costa *et al*., 2001; Lema-Rumińska and Fijałkowska, 2006).

The aim of the present study was to determine the effect of the location of the primary explant, derived from different zones of the main stem in cactus and young axillary shoots on the induction of direct somatic embryogenesis and the regeneration of shoots and callus. To our knowledge, the present study is the first report on obtaining somatic embryos in cacti *C. tenuissima* Ritt. f. *monstruosa.*

Materials and methods

Initial explants (mammillae with areoles) were taken from four donor plants of *Copiapoa tenuissima* Ritt. f. *monstruosa* grafted on the pad from genus *Cereus*; the plant material was obtained from the collection of Licznerski (Jarużyn Kolonia near Bydgoszcz). The explants were taken from three zones of the main shoot of donor plans: distal, central, proximal (Figure 1A) and from young axillary shoots (Figure 1B). They were sterilized with 70% ethanol for 1-2 s and then with 0.79% hypochloride solution for 15 min, followed by three rinses with distilled sterilized water. There were used 100 explants (in this some physiological stage) depending on the zone of donor plans and the medium. Explants were cultured on modified Murashige and Skoog (1962) medium (MS) with additional 330 $mg \cdot dm^{-3}$ $CaCl_2 \cdot 6H_2O$, 13.9 $mg \cdot dm^{-3}$ $FeSO_4 \cdot 7H_2O$ and 20.6 $mg \cdot dm^{-3}$ $Na_2EDTA \cdot 2H_2O$. The medium contained 3% sucrose, and it was solidified with 1.2% PURIFIED LAB-AGAR (Biocorp), pH was 5.7 prior to sterilization.

The explants were cultured on the modified MS medium with 2 $mg \cdot dm^{-3}$ auxin 2.4-D (2.4-dichlorophenoxyacetic acid) or MS medium without growth regulators (as control). The cultures were kept in a growth room at 24 ± 2 °C and exposed to 16 h photoperiod. Daylight was by maintained using Philips TLD54/36 W lamps with the photon flux density of 38.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After eight weeks of culture, the explants were examined under the stereomicroscope. The data were statistically analyzed by using the t-Student test, $p<0.05$.

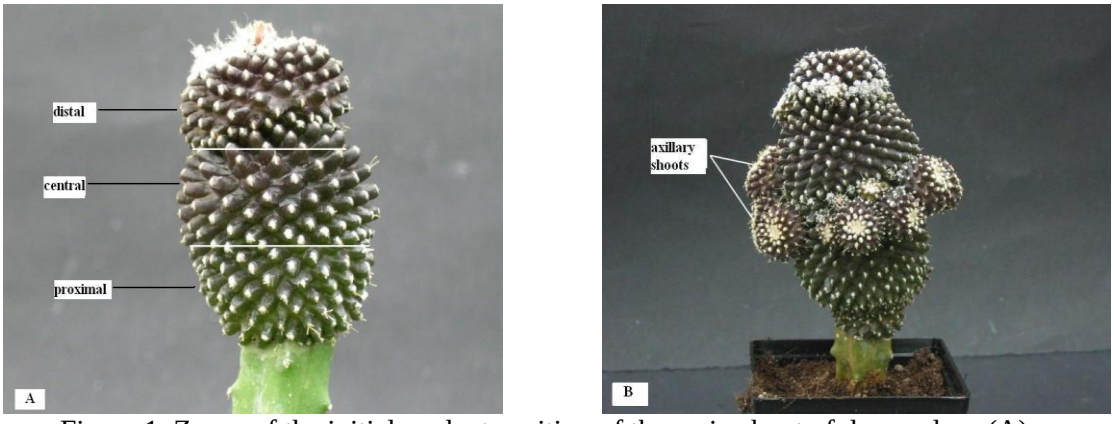

Figure 1. Zones of the initial explant position of the main shoot of donor plans (A) and young axillary shoots (B).

Histological analysis

Somatic embryos were immediately fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) buffer pH 7.2 overnight at 4 °C. After washing in PBS, the material was dehydrated in a series of increasing ethanol concentrations and then embedded in (butyl methacrylate, methyl methacrylate, 0.5% benzoin ethyl ether, 10 mM dithiothreitol; Fluka Chemie GmbH, Switzerland) resin (BMM). The embedded material was cut into semithin sections that were placed on Biobond-covered microscope slides (Niedojadło *et al*., 2008).

Results and discussion

The number of explants producing somatic embryos in *Copiapoa tenuissima* Ritt. f. *monstruosa* was significantly higher on the MS medium containing auxin 2,4-D than in the control MS medium, except for the

proximal zone where no differences were noted between the used media (Table 1). The greatest number of explants producing embryos was found from the middle part of the main stem (15%) and in young axillary shoots (13%) on the medium with auxin, respectively. Similarly, a high level of regeneration of somatic embryos was noted in cactus *Ariocarpus kotschoubeyanus* (Lem.) K. Schum. by Moebius-Goldammer *et al*. (2003); however, the medium for regeneration showed a high concentration of cytokinin BA (8.9-22.2 μM = 2.2-5.6 $mg \cdot dm^{-3}$) and auxin NAA at lower concentration (0.5–5.4 μM = 0.1-1.1 $mg \cdot dm^{-3}$). The somatic embryos obtained in *Copiapoa* were found at the globular stage cream-yellow in color (Figure 2), similarly as the embryos reported by Moebius-Goldammer *et al*. (2003). Lightly opalizing color of embryos demonstrates the accumulation of substance reserves, which points to their good quality (Cailoux *et al*., 1996, cited after Gomes *et al*., 2006).

Many authors point to the determining role of auxins in induction of somatic embryogenesis (Infante, 1992). Gomes *et al.* (2006) found that 1-4 $mg \cdot dm^{-3}$ Picloram induces successfully direct regeneration of somatic embryos from shoot apices in *Opuntia ficus-indica* (L.) Mill. Our results also confirmed the essential role of auxin 2.4-D (2 $mg \cdot dm^{-3}$) on the direct induction from areoles of mammillae, somatic embryogenesis in cactus *Copiapoa tenuissima* Ritt. f. *monstruosa*.

However, Gomes *et al*. (2006) suggested that auxin does not induce the process of somatic embryogenesis but only stimulates it, and the extremely stress is responsible for induction, which demonstrate higher numbers of regenerated embryos from damaged growth tops towards intact shoot apices. Besides, Gomes *et al*. (2006) also pointed out an important role of physiological stage of the explant prior to the stress treatment, which may be playing a more decisive role in the induction of somatic embryogenesis than the extremely applied growth regulator. They found that younger apices in *Opuntia ficus–indica* (L.) Mill. are better suited for somatic embryogenesis than the older ones. The physiological state of the explant and its topophysical location on the mother plant also determined the pattern of the induction of somatic embryogenesis in cactus *Copiapoa tenuissima* Ritt. f. *monstruosa*. It was found that most somatic embryos regenerated in the explants derived from the distal and central zone of the main stem and from the explants derived from young axillary shoots (Table 2). However, it was noted that the lowest number of somatic embryos regenerated from the explants corresponded to the proximal zone of the main stem. Besides, somatic embryos on the MS medium with 2.4-D on the explants derived from the distal level also regenerated few axillary stems (Table 2); in addition, a similar number was found on the control MS medium on the explants sampled from the distal level of the main stem and in young lateral shoots. Few calluses were formed on explants, irrespective of the level of its sampling.

The location of the explant on the plant during micropropagation in some topophysis-dependent cultivars in *Chrysanthemum* x *grandiflorum* Ramat./Kitam. can determine many characters, e.g. propagation rate, growth rate, shoot length and it was higher, when the explants derived from the central and proximal zones of the plant (Zalewska *et al*., 2010). Additionally, there was investigated the effect of the position of the explant and its age on the development of axillary shoots in *Rosa hybrida* plants (Bredmose and Hansen 1996; le Bris *et al*., 1998). Moreover, the effect of the explant position on the donor plant on the induction of somatic embryogenesis was investigated in *Capsicum annuum* L. (Kintzios *et al*., 2000) and in *Zamioculcas zamiifolia* Engelm. (ZZ) (Papafotiou and Martini, 2009). It is therefore probable that the location of the explant on the mother plant and its physiological condition determined topophysically can also determine the pattern of the process of induction of somatic embryogenesis in cactus of *Copiapoa* genus.

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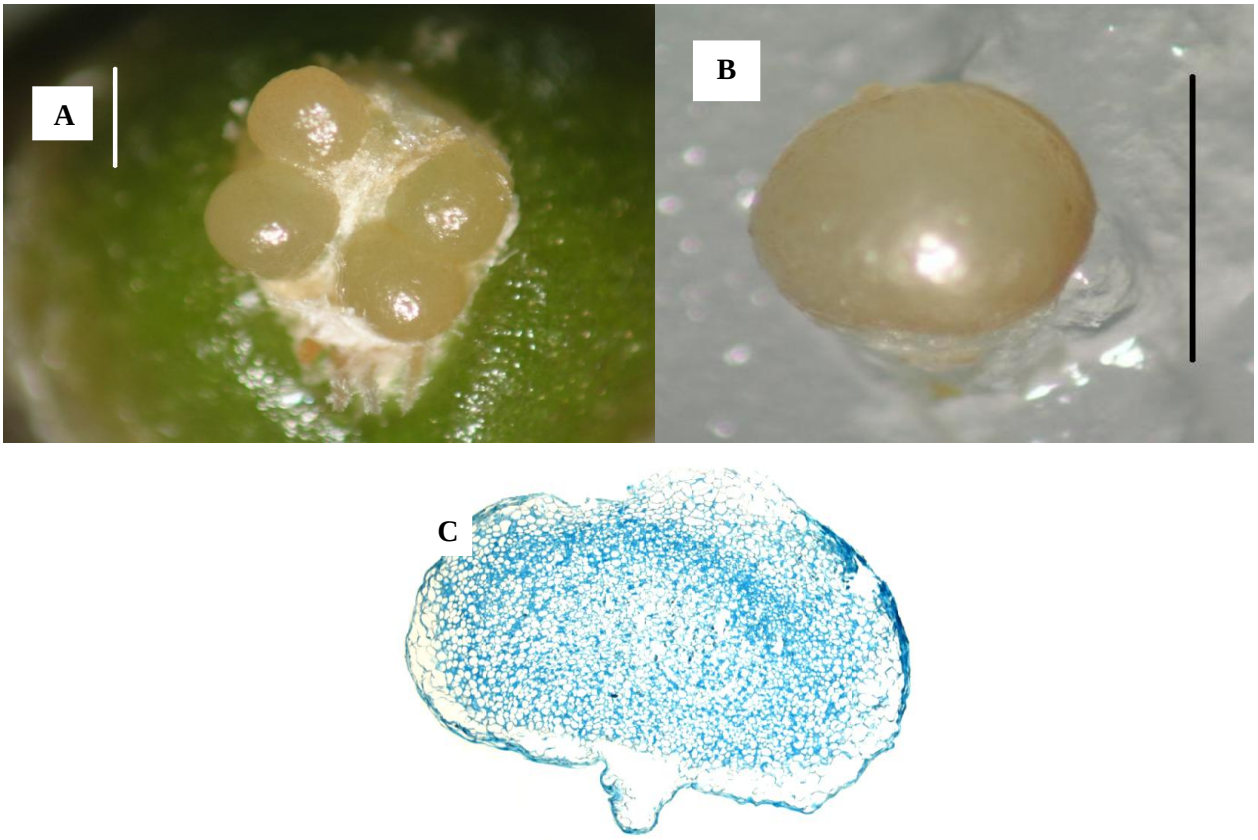

Figure 2. Globular stage of somatic embryo (A, B) and its histological analysis (C) in *Copiapoa tenuissima* Ritt. f. *monstruosa* (1 bar = 1 mm).

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Table 1. Number of explants producing somatic embryos depending on the kind of the medium and the zone of explant sampling in cactus *Copiapoa tenuissima* Ritt. f. *monstruosa*

Zone of explant sampling		Medium	
		MS + 2,4-D	MS (control)
Distal	Main shoot	0.10Aac*	0.00 B
Central		0.15 Aa	0.00 B
Proximal		0.02 Abc	0.00 A
Axillary shoot		0.13 Aa	0.00 B

* a, b: Data in columns marked with the same lower-case letter do not differ significantly at α=0.05
A, B: Data in lines marked with the same upper-case letter do not differ significantly at α=0.05

Table 2. Number of regenerated embryos, shoots and callus depending on the zone of explants sampling in cactus *Copiapoa tenuissima* Ritt. f. *monstruosa*

Zone of explant sampling		Medium											
		MS + 2,4-D						MS (control)					
		embryos		shoots		callus		embryos		shoots		callus	
		total	on one explant	total	on one explant	Total	on one explant	total	on one explant	total	on one explant	total	on one explant
Distal	Main shoot	24	0.24 abB*	8	0.08 aB	11	0.11 aA	0	0.00 aB	16	0.16 aB	3	0.03 aB
Central		17	0.17 aA	1	0.01 aB	9	0.09 aA	0	0.00 aB	0	0.00 aB	0	0.00 aB
Proximal		2	0.02 bB	0	0.00 aB	3	0.03 abA	0	0.00 aB	0	0.00 aB	0	0.00 aB
Axillary shoot		26	0.26 abB	0	0.00 aB	3	0.03 bB	0	0.00 aB	16	0.16 aA	2	0.02 aB

* a, b: Data in columns marked with the same lower-case letter do not differ significantly at α=0.05
A, B: Data in lines marked with the same upper-case letter do not differ significantly at α=0.05

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