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Realisation of the morphogenetic potential of some *Scilla* L. species *in vitro*

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Summary: The results of a comparative morpho-anatomical study of regeneration in bulb scale and leaf explants of 3 species of *Scilla* are presented. The early stages of morphogenesis, peculiarities of initial cell divisions, differentiation of hydrocyte system and adventitious structures and cork tissue formation are described, features of similarity and specific differences were revealed. Practically, all living tissues of explants display regeneration activity. Numerous cell divisions lead to the formation of extensive meristematic zones, where bud and root rudiments differentiate.

Keywords: *Scilla*, bluebell, regeneration, morphogenesis *in vitro*, polyads, meristematic clumps, hydrocyte system, shoot and root rudiments

Representatives of the genus *Scilla* L.(Asparagaceae) are early spring flowering small bulbous plants which are widely used in landscape gardening due to their ornamental appearance. These bulbous ephemeroids emerge soon after snowbreak and are flowering during 3 weeks, when other perennials just begin to sprout. They are winter-hardy and withstanding to very unfavourable factors (low temperatures, high soil and air humidity), which are common in early spring. Some species are cultivated as indoor plants. Only few bluebell species are listed in Red Data Books or lists of threatened plants (IUCN 2021).

In this paper, the results of studying morphogenetic processes in some bluebell species are presented. Inspite of extensive practical application of microclonal propagation of monocotyledonous plants and numerous articles on this matter, the information on anatomy of morphogenesis *in vitro* is still very scarce. In the available literature on morphogenesis of bluebell tissue culture (Hussey 1975; Yanagawa & Sakanishi 1980; Chakraborty & Sen 1983; Crouch et al. 1999; Ozdemir et al. 2016), main attention is paid to its hormonal regulation, selection of different composition of planting media and some physiological and biochemical aspects as well. Unfortunately, neither photographs nor drawings illustrate the different stages of morphogenesis which are of our interest in the aspect of realisation of morphogenetic potentialities of different plant organs.

Thus, our study was aimed at elucidation and analysis of totipotent features of cells from different tissues, scarring of wounds, analysis of histological changes during regeneration, reproduction of adventitious structures and hydrocyte system development. Main attention is paid to the analysis of morphogenetic processes in the explants of vegetative organs of the same morphological nature (bulb scale and leaf), but of different structure and functional loading.

Materials and methods

The objects of our investigation were three species: *Scilla sibirica* Andr., *S. rosenii* C. Koch. and *S. italica* L. (syn. *Hyacinthoides italica* (L.) Rothm.).

Plant material was submitted to presterilisation with Fundazol and to subsequent sterilisation. The methods were described in detail earlier (Churikova et al. 1991). After sterilisation, the explants (segments of young medial leaves and bulb scales) were placed onto basic nutrient medium MS according to Murashige & Skoog (1962) with the addition of 5 mg/l BAP, 1 mg/l NAA and 30 g/l sucrose. The preparation of culture mediums, presterilisation manipulations and sterilisation of plant material were conducted according to Rumynin & Slyusarenko (1989). The methodology of plant culture *in vitro* and preparation of samples for microscopic investigations followed Churikova et al. (1991). Anatomical sections of three leaf formations were analysed using light microscope Micromed-3. Images of the sections were taken with light microscope Axioplan-2 Imaging equipped with digital camera AxioCam MRc and processed with Adobe Photoshop.

Results and discussion

The different functional loading of assimilative leaves and bulb scales, which were used as a resource of explants, affect some qualitative and quantitative features of anatomical structure (Table 1). Storage bulb scales consist of 23–27 layers of rounded, thin-walled cells. Parallel vascular bundles run through the bulb scales. Their number differs from 4–5 (*S. rosenii*) up to 15–18 (*S. italica* and *S. sibirica*), and they have well noticeable parenchyma sheaths. The main storage substance is starch, cells with raphides of calcium oxalate are found from time to time. The epidermis of adaxial and abaxial surface of bulb scales consists of small rather thin-walled cells.

Leaf blades are relatively thin, with 13–25 layers of homogeneous mesophyll. Its peripheral cells contain numerous chloroplasts. The number of vascular bundles is similar to that of bulb scales (*S. rosenii* and *S. italica*) or twice more (*S. sibirica*). Epidermal cells of morphologically upper and lower surface have feebly thickened external and relatively thin radial cell walls.

Table 1. Quantitative characteristics of anatomical structure and some peculiarities of early morphogenetic processes in bulb scale and leaf explants of *Scilla sibirica*, *S. italica* and *S. rosenii in vitro.*

Species	Thickness (number of cell layers)		Number of vascular bundles		Raphids		Cell divisions				Presence of hydrocyte nodules and bundles				Site of apex initiation			
							I		II		I		II		I		II	
	I	II	I	II	I	II	ad	ab	ad	ab	ad	ab	ad	ab	ad	ab	ad	ab
Scilla sibirica	13–14	37	15–17	7–8	-	+ few	+ subep	+	+ subep	+	-	-	-	+	sh subep rt clad	-	sh subep	rt
Scilla italica	23–25	35	15–18	15	+ many	+ many	+	+ (at first)	+	+ (at first)	+	+	-	+	sh	rt	sh	rt
Scilla rosenii	17	23–35	4–5	4–5	+	+	+	+ (at first)	+	+ (at first)	-	-	-	+	sh	+	sh subep	rt

I – leaf; II – bulb scale; ab – abaxial side of the leaf or bulb scale; ad – adaxial side; rt – root; rt clad – cladogenic root; sh – shoot; subep – cell divisions in subepidermal layer.

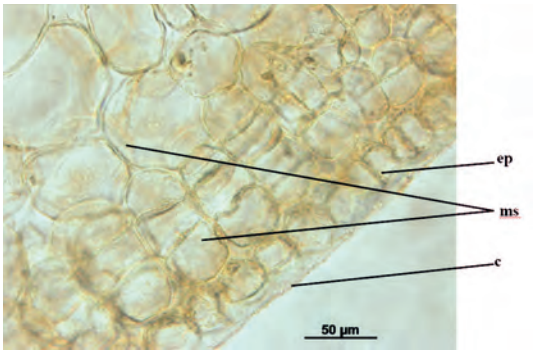

Figure 1. The first periclinal cell divisions near the abaxial side of *Scilla italica* bulb scale explants: c – cuticle; ep – epidermis, ms – mesophyll.

Our results demonstrate that mophogenetic processes occur in similar manner in all three bluebell species. However, the highest regenerative activity is characteristic of *S. italica*. Cell divisions in leaf and bulb scale explants begin on the 10th–12th day of cultivation and are localised in subepidermal layers near the abaxial side. It takes place because of their direct contact with the nutrient medium. Outwardly, it reveals in the formation of swelling near the cutted surface. The first cell walls are periclinal (Fig. 1). However, as we noticed earlier for lilies and hyacinths (Churikova & Barykina 2015), such regular manner of cell divisions soon gets lost. A group of small cells with dense cytoplasmic content and well distinguishable large nuclei (polyad) develops within the common initial cell membrane (Fig. 2A, B). Later on, a common meristematic zone is formed due to the lysis of cell walls of several nearby located polyads. Epidermis, as a rule, doesn't take part in its formation immediate. Cell divisions involve deeper mesophyll layers gradually (Fig. 6A). So, more and more alive cells including the cells of parenchyma sheaths of vascular bundles are involved in meristematic activity. At this time, the first cell divisions of 1–2 subepidermal layers of the adaxial side take place. As a result, two oncoming waves of cell divisions appear and gradually disappear in the central part of the explant. In spite of the relatively later meristematic activity of the cells of the adaxial side, the reproduction of adventive structures is more extensive there (Fig. 3). On the contrary, early appearing meristematic clumps on the abaxial side, as a rule, don't differentiate into shoot apexes. Shoot rudiments always develop endogenously (Fig. 4A). Later on, root initials form in the base of the shoot apexes.

At the same time, when such cladogenous roots were formed, we noticed the development of root rudiments independently from buds. They appear in deep layers of divided mesophyll cells from

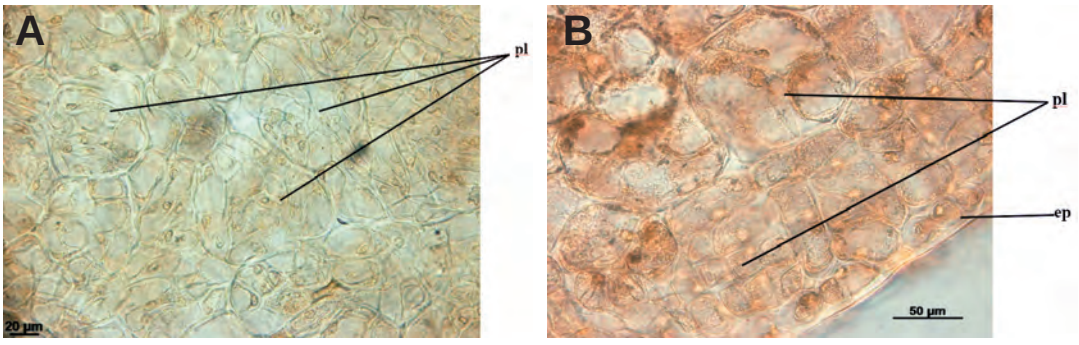

Figure 2. Formation of polyads in *S. italica* (A) and *S. rosenii* (B) bulb scale explants: ep – epidermis; pl – polyads.

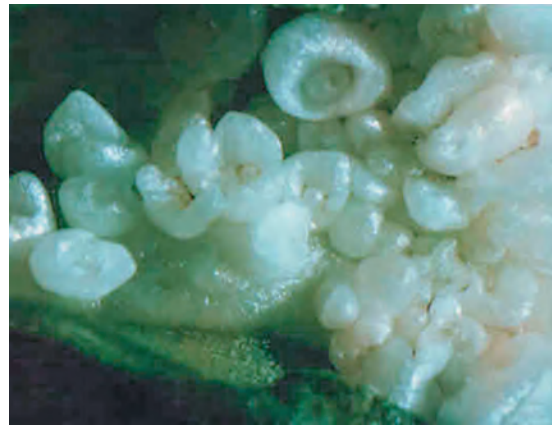

Figure 3. *De novo* formed bulblets in *S. sibirica* bulb scale explants.

the abaxial side. The formation of shoot apexes (Fig. 4B) is often preceded by their development. During their growth, the root rudiments move peripheral mesophyll layers with epidermis back and get in touch with the nutrient medium (Fig. 5).

Quite often, the formation of an extensive hydrocyte system in the common meristematic zone of the explant precedes the emergence of shoot and root rudiments. It is represented by numerous hydrocyte nodules and relatively wide 'cross-pieces' which include vascular elements of phloem and xylem and connect the hydrocyte nodules with each other (Fig. 6B). Hydrocyte nodules often become peculiar initial centers of further morphological processes which lead (in turn) to bud development. The revealed, more intensive hydrocyte system formation in bulb scales compared to leaves leads up to increased activity of regenerative processes here. Once again developed, such vascular system of explants takes part in the redistribution of nutrient substances and evidently in stimulation of meristematic activity of cells and in regulation of further morphogenetic processes (Churikova & Barykina 2005). The correlation between the degree of hydrocyte system development and the number of shoot rudiments appearing *de novo* is an indirect confirmation of above mentioned matter.

As to the specific peculiarities of regenerative processes in studied bluebell species, they mainly reveal themselves in the character of healing wounded explant surface. So, suberinated cell walls

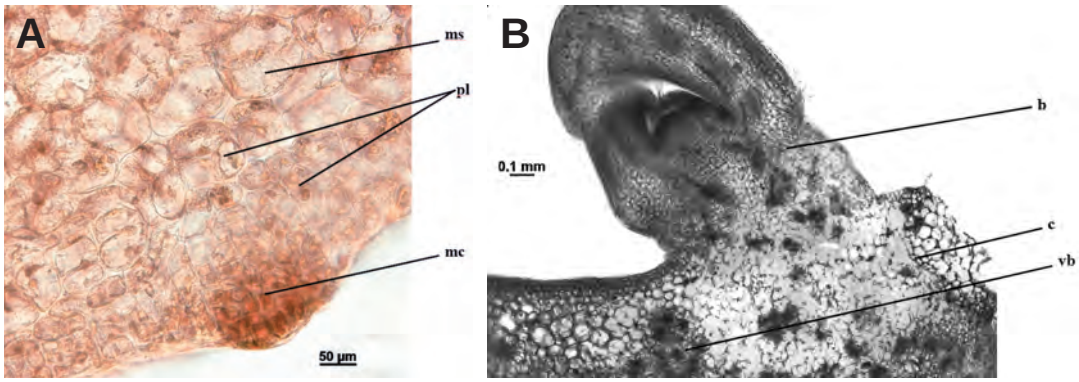

Figure 4. Early stages of shoot rudiment development (A) and formation of bud (B) in *S. rosenii* bulb scale explant: b – bud; c – wound cork; mc – meristematic clump; ms – mesophyll; pl – polyads; vb – vascular bundle.

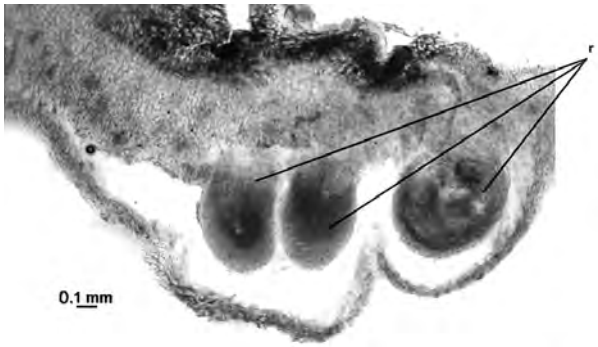

Figure 5. Independent root rudiments (r) formation in *S. italica* bulb scale explant.

of living tissue cells without phellogen formation near the cut surface is characteristic of *S. italica.* In *S. rosenii*, superficial wound cork includes few cell layers (Fig. 7A, B). *Scilla sibirica* differs in deeper formation of multilayered, greatly suberized wound cork.

Thus, the analysis of regenerative processes in the explants of leaf nature in three bluebell species showed that practically all living tissues of explants (mesophyll, storage parenchyma, sheaths of vascular bundles) display regenerative activity. Epidermis is an exception, its cells undergo only periclinal divisions, but don't take part in meristematic clumps formation immediate. So, our data concerning totipotency of epidermal cells during the reproduction of adventitious organs *in vitro* differ from the results of Yarvekulg (1965). He studied the regeneration of *S. sibirica* leaf grafts *in vivo*, in moist sand. According to Yarvekulg (l. c.), adventitious bulblets always appear exogenously from epidermal cells of the adaxial surface. It cannot be excluded that high totipotency of epidermal cells in this case is connected with their weak differentiation near the intercalary zone of leaf grafts which were used. Relatively weak regenerative activity of epidermis in our experiment apparently is due to its early and deep specialisation compared to mesophyll, early loss of cell's ability to renew meristematic activity and lack of sufficient energetic resources (nutrient substances) as well.

Intensive formation of hydrocyte system is one of the most dramatic histological changes in explants during regeneration. A well-developed hydrocyte system, especially in bulb scales, provides for transmission and mobilisation of energy-rich and biologically-active substances,

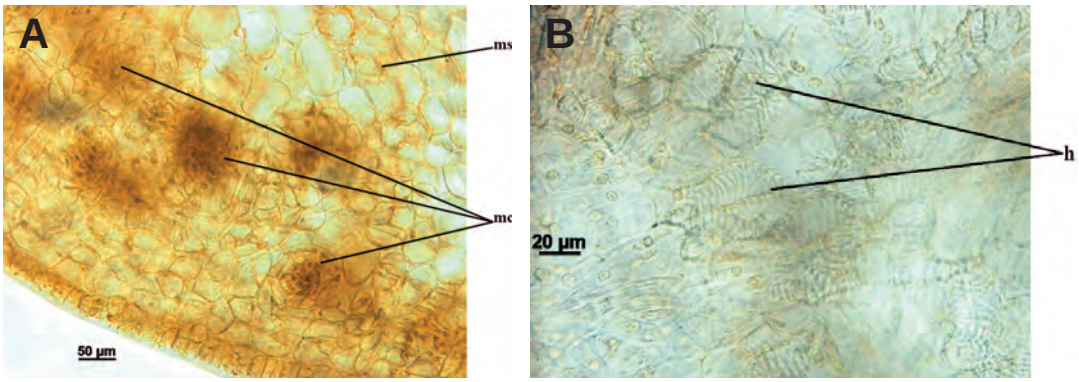

Figure 6. Numerous meristematic clumps (A) and first stages (B) of hydrocyte's formation in *S. italica* bulb scale explant: h– hydrocyte; mc – meristematic clump; ms – mesophyll.

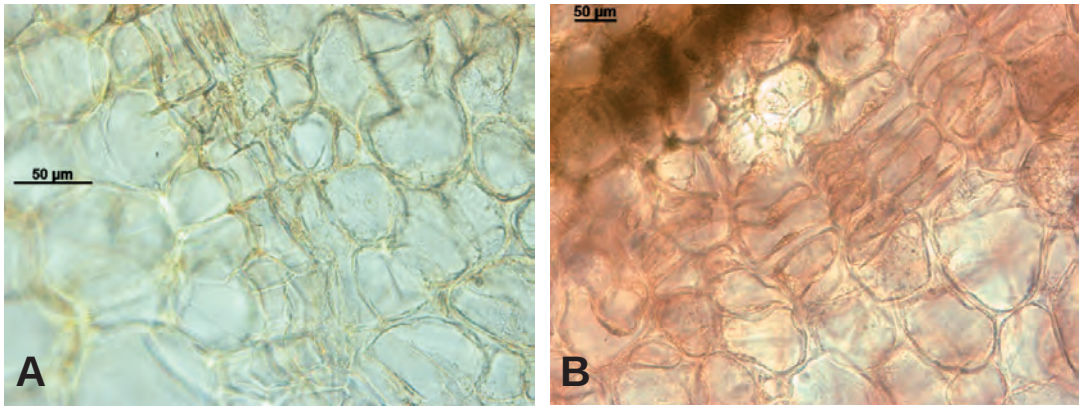

Figure 7. Wound cork in *S. italica* (A) and *S. rosenii* (B) bulb scale explants.

which are necessary for differentiation and further development of de novo formed bud and root rudiments.

Bud rudiments are of exclusively endogenous origin. They quickly increase the number of metameres and roots relatively early. Their endogenous origin provides for defence and better nutritive conditions of *de novo* developed structures.

High regeneration capacity to reproduction of adventitious structures in studied bluebell species tissue culture evidently is characteristic of other species as well, including rare and endangered ones. In this regard, the results of our investigation may be applied for improvement of the microclonal propagation protocol for *Scilla* species and other small-bulbous plants.

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