

The 'hydrocyte system' in seed plants

Olga A. Churikova & Rimma P Barykina

Summary: Formation of the hydrocyte system can be found very often in seed plants. In particular, during the formation of root shoots from adventitious buds in rootstock plants, in the case of natural coalescence of roots, and in different methods of shoot grafting. More strikingly it becomes apparent during regeneration; both *in vivo* and *in vitro*. The intensity of its development mainly depends on the organ's morphological nature, its functional loading and to an even lesser degree on the taxonomical affiliation. Detailed analyses of the hydrocyte system at light and electron microscopical levels show its complex histological structure. It includes water-conductive elements (hydrocytes-tracheids, rarely vessel members), phloem elements (sieve cells and sieve tube members with companion cells) and tracheidal parenchyma cells. The latter accomplish functions of transferring, supporting, accumulation and storing of reserve substances. Therefore, the so-called 'hydrocyte system' can be regarded as an original variety of the conductive system of seed plants.

Keywords: seed plants, morphology, vegetative organs, anatomy, hydrocyte, vascular system differentiation *in vivo* and *in vitro*

The method of plant microclonal propagation *in vitro* is a very modern method of experimental biology with a promising outlook for the future. That's why it is rapidly developing nowadays. During the propagation process in plant tissue culture, a special type of cells with lateral cell wall thickening can be observed often. These cells, called 'hydrocytes' (PERVUCHINA 1945; ALEXANDROV 1954), form compact nodules or elongated bundles. In botanical literature, especially the Russian, the terms 'hydrocyte bundles' and 'hydrocyte nodules' are widespread.

The hydrocyte system is a relatively frequent structure in flowering plants. Its presence was discovered in plants of different growth form, i.e. herbs, shrubs, and trees, especially often in the case of vegetative propagation. In particular, it can be discovered during the formation of root shoots from adventitious buds of rootstocks (RAUH 1937), in the case of natural coalescence of roots in various angiosperms (LOTOVA & LIARSKAYA 1959) (fig.1), and in different methods of shoot grafting (ALEXANDROV 1954). For example, its formation can be observed in *Robinia pseudoacacia* during the development of adventitious buds in deeper tissues. The buds stay resting over a long period of time. Until their appearance at the surface of the organ they grow within the tissue of the node. The development of the hydrocyte system is especially evident when buds are formed in superficially situated organ parts and in callus. Some authors (HUBER 1947; LEDERER 1955; GRIFFITH 1957; YATSENKO-KHMELEVSKY 1961) describe some elements of a hydrocyte system also in gymnosperms. In particular, YATSENKO-KHMELEVSKY (1961) notes its presence in transfusion-tissue. Besides, hydrocytes develop in reproductive organs as well. For example, they were observed in the pericarp of some Asteraceae (ALEXANDROV 1954), Brassicaceae (KANIEWSKI 1967), and Fabaceae (KANIEWSKI & WAŻYŃSKA 1968).

The formation of a hydrocyte system is an always appearing phenomenon in tissue culture *in vitro* when organs of different morphological nature are used as explants (e.g. foliage leaves, scalelike leaves, peduncles). We found the most strongly developed hydrocyte system in tissue

culture by using stored scales as explants. The intensity of hydrocyte system development mainly depends on the morphological nature of the organ and its function. To an even lesser degree, it depends on taxonomical affiliation. The hydrocyte system is responsible for the transport of energy-rich substances and hormonal regulators to sites of meristematic activity.

In spite of the great interest to the problem of vascular system differentiation, both *in vivo* and *in vitro* (e.g. BUTENKO 1964; ROBERTS 1976; BOLWELL 1989), its histological structure was not investigated in details up to now. Only the very general descriptions of principal structural elements of the hydrocyte system were published (l.c.). In particular, the presence of water-transferring elements – hydrocytes (hence the term ‘hydrocyte system’ appears) is noted and adjacent to them live elements, which provide the transfer of energy-rich substances.

Since the hydrocyte system remains insufficiently described, we are presenting now its detailed structural analysis at light- and electron-microscopical levels. Apart from original data, we summarize our previous publications on the origin of adventitious buds on the roots of different members of herbaceous, shrubby and arboriform plants *in vivo* (BARYKINA 1954, 1958; BARYKINA & LOTOVA 1962) and their formation in explants of different vegetative organs of some bulbous and cormous monocotyledons *in vitro* (CHURIKOVA et. al. 1991, 1994; CHURIKOVA & BARYKINA 1995; BARYKINA & CHURIKOVA 2001).

Materials & methods

The objects of our investigations were dicotyledonous trees and shrubs (*Hippophae* sp., *Malus* sp., *Phellodendron* sp., *Robinia* sp.), taken out from natural habitats and monocotyledonous herbs (*Lilium regale* Wils., *L. longiflorum* Thunb., *L. speciosum* Thunb., *L. pardalinum* Kellogg., *L. candidum* L., *L. martagon* L., *Hyacinthus orientalis* L., *Muscari* sp., *Scilla* sp., *Gladiolus hybridus* hort., *Narcissus hybridus* hort.) which were cultivated *in vitro*. Detailed descriptions of explants and protocols for nutrient mediums preparing, were published earlier (RUMYNIN & SLYUSARENKO 1989; CHURIKOVA 1993). The morpho-anatomic analysis was carried out according to established methods (BARYKINA et. al. 1971, 2004). To study the anatomy of explants, we prepared longitudinal and cross sections. Cell walls of conductive elements were investigated by means of transmission electron microscope (JEOL JEM-100B-2).

Results & Discussion

As a result of our investigations, we can show that hydrocyte nodules are formed very early: during the very first cell divisions, which result in development of polyads. These are complexes of divided cells within the common initial cell membranes (fig. 2a). These nodules act as centers of further morphogenetic differentiation (fig. 1a,b).

Hydrocyte nodules often locally differentiate in the centre of the common subepidermal meristematic zone. From these nodules, the formation of vascular elements starts in two opposite directions: acro- and basipetal. As a result the continuous connection of the vascular systems of the de novo developing adventitious buds and the maternal organ arises (fig.1d).

At least, the development of the hydrocyte nodules can precede the endogenous differentiation of shoot apices from the more deeply situated embryonic complexes. Our investigations of vegetative organ explants of some bulbous and cormous monocotyledons, focusing on surgical

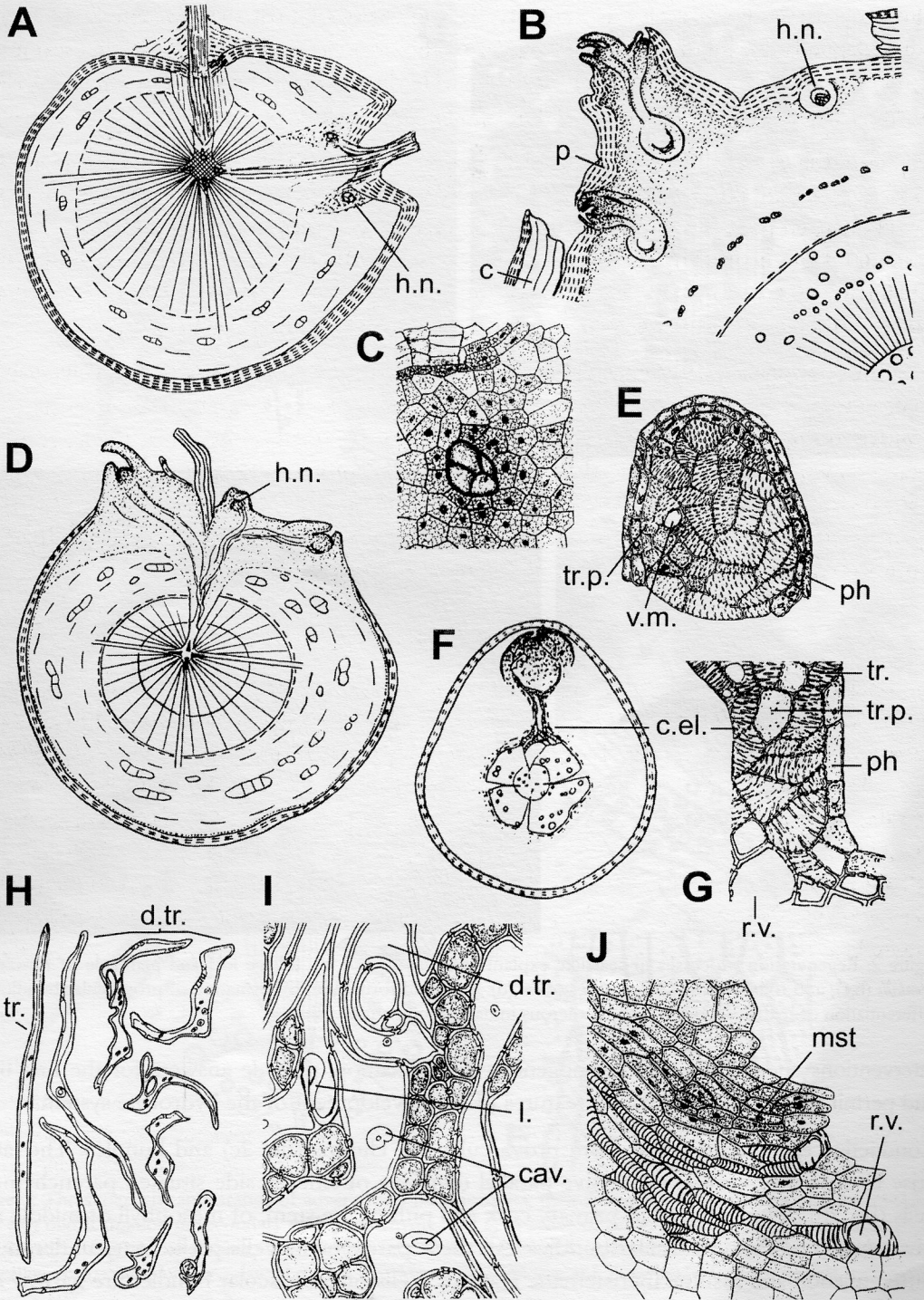


Figure 1: The hydrocyte system within the differentiation and development of adventitious buds on the roots of Dicotyledons *in vivo* (a *Populus alba*, b,c *Elaeagnus angustifolia*, d *Populus nigra*, e-g *Phellodendron amurense*, j *Phellodendron deltooides*), and tracheids at the loci of coalescent roots of *Cedrus deodorata* (h) and *Cedrus atlantica* (i). c – cork, cav. – isolated cavities inside the tracheid, c.el. – conductive elements at the base of the bud, d.tr. – distorted tracheids, h.n. – hydrocyte nodule, l. – loop inside the tracheid, mst – young member of sieve tube, p – periderm, ph – phloem, r.v. – vessel of the root, tr. – tracheids, tr.p. – tracheidal parenchyma, v.m. – vessel member.

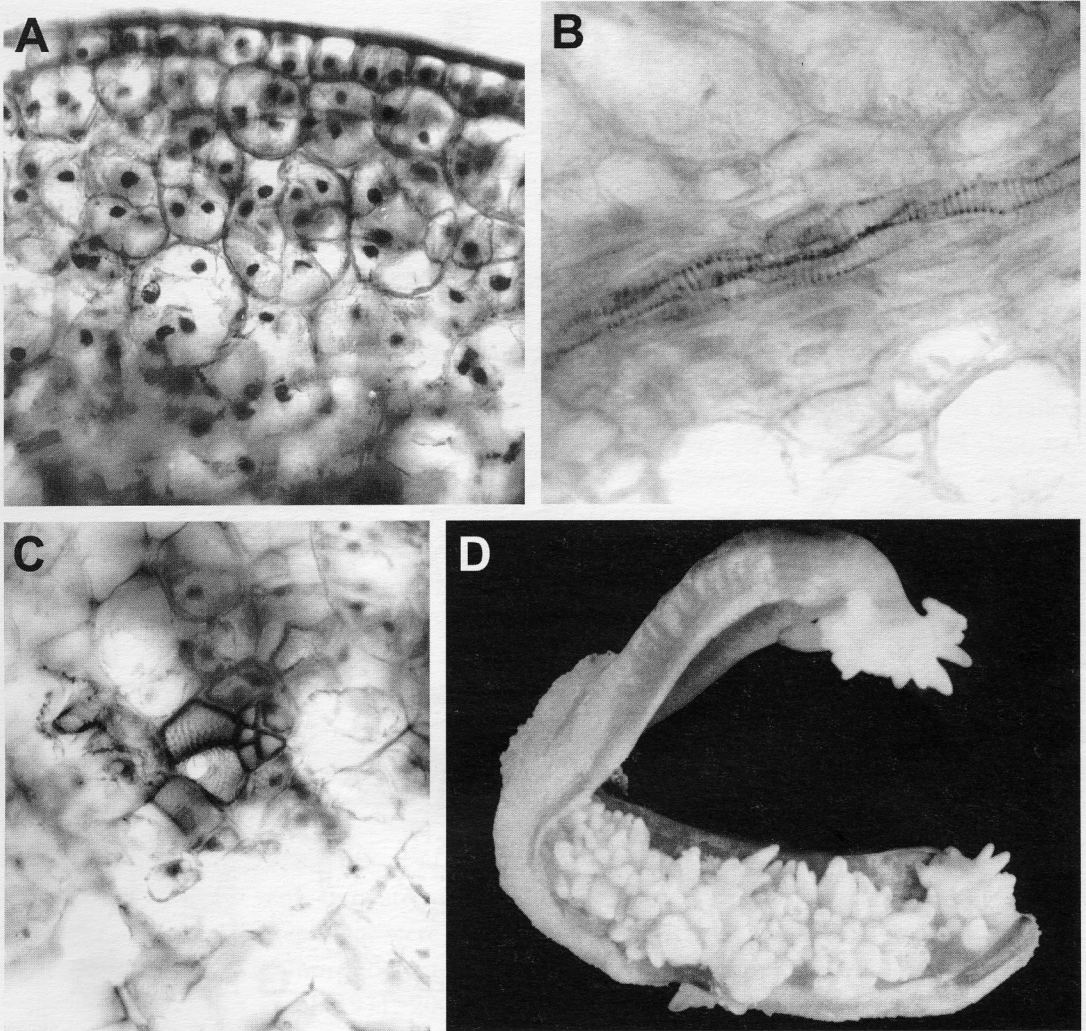


Figure 2: Regeneration processes in scalelike explants of *Lilium regale* (a), foliage leaf and peduncle of *Hyacinthus orientalis* (b,c), and foliage leaf of *Muscari racemosum* (d). a) polyad formation, b) formation of provascular bundles, c) differentiation of hydrocyte nodules, d) numerous initials of adventitious shoots.

interventions and successive morphogenetic differentiation, provide answers for the first time and permit to reveal the structural features of the development of the hydrocyte system.

Conducting elements develop from provascular cell clumps (fig. 2c) and bundles. The latter arise by means of several successive parallel divisions of side by side situated parenchymatic cells (fig. 2b) of inner layers of primary cork and pith of the stem, of mesophyll of middle and lower leaves of facing vascular bundles. All these parenchyma cells preliminary undergo de-differentiation and assume meristematic features. Cells of provascular bundles are narrow and relatively short, sometimes they are stretched longitudinally. They have dense cytoplasmatic contents and large nuclei.

The xylem-part of the hydrocyte system is studied more in details. Its basic water-transferring elements are hydrocytes: tracheids with different types of lateral cell wall thickening (spiral, retinal, spotted) and bordered pores (fig. 1h,i). They keep the live cytoplasmic contents and

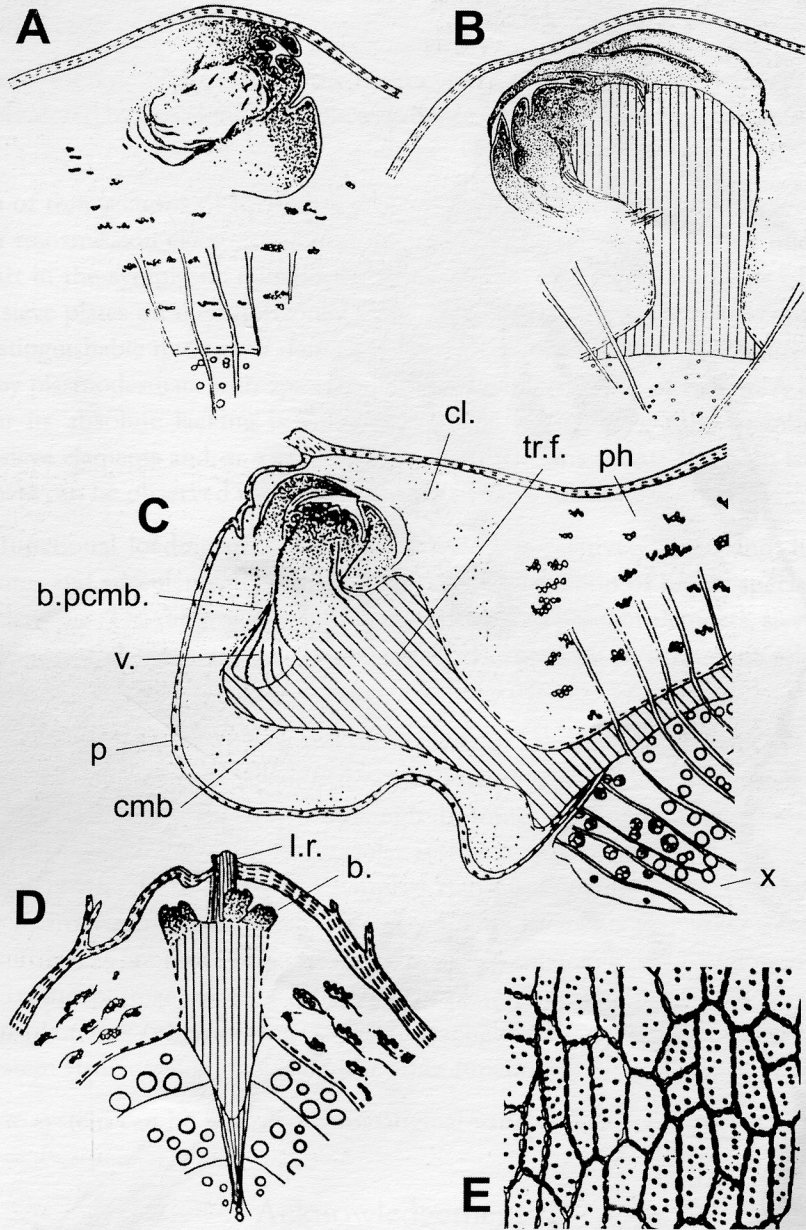


Figure 3: The superficial appearance of adventitious buds in roots, their formation and structure of the tracheidal foot. a,b) bud formation in secondary cork parenchyma, and c) in callus of *Malus* species, d) serial bud appearance near the adsorbing root of *Robinia pseudoacacia*, e) cells of the tracheidal foot. b. – bud, b.pcmb. – bud procambium, cl. – callus, cmb – cambium, l.r. – lateral root, p – periderm, ph – phloem, tr.f. – tracheidal foot, v – vessels, x – xylem.

cell nuclei for a long time. Side by side with them, short vessels with simple perforations on their lateral cell walls (BARYKINA & LOTOVA 1962) and tracheidal parenchyma cells can be found (fig. 1e,g). The latter accomplish the functions of transferring, supporting, accumulation and storing of reserve and refuse substances. Their cell walls undergo the lignification process and possess simple and also bordered pores.

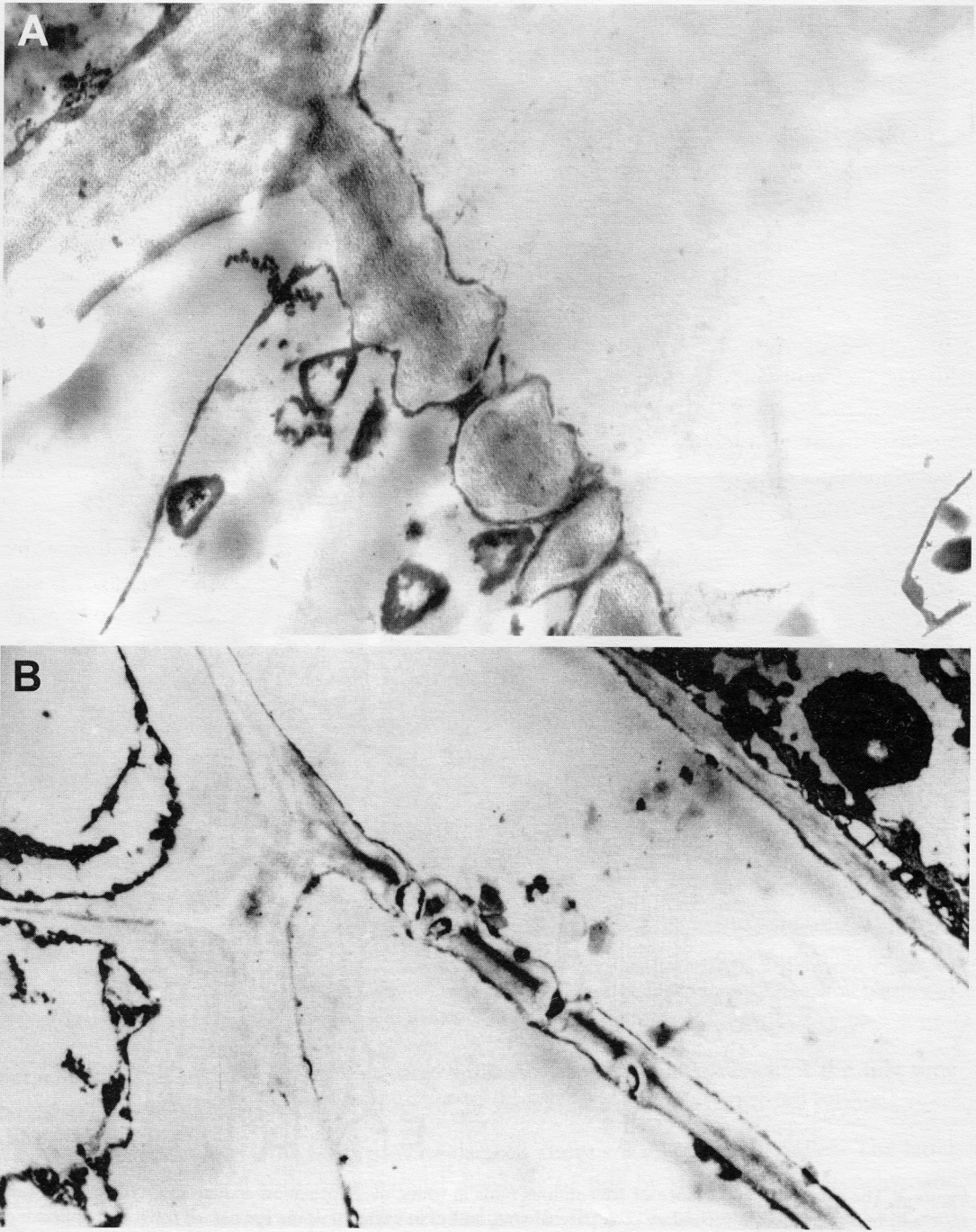


Figure 4: Photographs of sections of a *Narcissus hybridus* peduncle by means of transmission electron microscopy.

As an example of considerably developed tracheidal parenchyma, we show parenchyma in roots of some *Malus* (fig. 3a,b,c) (LIARSKAYA 1957) and *Robinia* (fig. 3d,e) (BARYKINA 1958) species. It is connected with slow apical growth of buds. They arise early in maternal root tissues and force their way to its surface during several years. The large cells of 'medullary' rays participate in this process. As a result, they acquire the appearance of an original 'foot' (fig.

3b,c,d). The vessel system of such buds is formed slowly, its connection to the root conductive system establishes late and is carried out by conductive elements which appear in the midst of the parenchyma cells.

In contact with the xylem elements of the hydrocyte system the phloem elements are formed. They are represented by live elongated cells with dense cytoplasm which are shorter in hydrocyte nodules.

The analysis of thin sections of hydrocyte adjacent cells in pedicle explants *in vitro* of *Narcissus* by means of transmission electron microscope, shows that, according to their microstructure, they take part in the symplastic transport of substances; i.e. they are segments of sieve tubes with simple sieve plates on their horizontal walls (fig.4a). Adjacent to them are companion cells with well distinguishable nuclei and dense cytoplasmic contents (fig.4b). Mature sieve plates are pierced by plasmodesmata with apertures lined by a little amount of callose. A little amount of callose or its absolute lacking is characteristic for young sieve tube members, actively functioning sieve elements and, as a whole, for monocotyledons (ESAU 1980). In isolated cases, plasmodesmata can be observed in lateral cell walls of sieve tubes.

In vitro the functional loading of conductive elements is relatively small and, like in water plants, parasites and succulents, there is no necessity in formation of highly specialized water-conducting elements. The differentiation of highly specialized phloem elements, sieve tubes with simple sieve plates and companion cells, apparently is caused by an important role of energy-rich and hormonal substances to realize a successful regeneration process.

Thus, the results of our investigations and the analysis of literature data permit the conclusion, that the development of the so-called 'hydrocyte system' is genetically programmed. It rather often occurs in seed plants, and most strikingly becomes apparent during the regeneration both *in vivo* and *in vitro*. It is a complex vascular system in structural and in functional respect, and provides very first the apoplast and symplast transport of substances. Its water transport elements are hydrocyte-tracheids, rarely vessel members. Elements providing the transport of energy-rich substances are more often sieve tubes with companion cells (Angiospermae), rarely sieve cells (Gymnospermae) or cells without sieve plates, but with numerous plasmodesmata and albumen filaments (phytoparasites). Alive tracheidal elements can be members of the hydrocyte system structure and they carry out other functions.

The hydrocyte system can be regarded as an original variety of the conductive system of seed plants.

Acknowledgements

The work was supported by the President of Russian Grant for Leading Scientific Schools (Project No. 1898.2003.4).

References

- ALEXANDROV, V. G. (1954):** Anatomy of plants. – Moscow: Gosizdat. Sov nauka. [In Russian]
BARYKINA, R. P. (1954): The formation of root stocks in some trees and shrubs, used in steppe afforestation for the soil strengthening. – Ph.D. Thesis, Lomonosov Moscow State University: Moscow. [In Russian]

- BARYKINA, R. P. (1958):** The specificity of root stocks formation in *Hippophae rhamnoides* L. – Bulletin Moskovskogo Obschestva Ispytatelei Prirody. Biologia **63**(4): 57–71. [In Russian]
- BARYKINA, R. P. (1960):** On the morphogenesis of bird-cherry tree underground reproductive organs. – Biologicheskiye nauki. **4**: 139–144. [In Russian]
- BARYKINA, R. P. & CHURIKOVA, O. A. (2001):** The peculiarity of morphogenesis in some *Scilla* L. species *in vitro*. – Vestnik Moskovskogo Universiteta. Seriya 16, biologiya **1**: 20–23. [In Russian]
- BARYKINA, R. P. & CHURIKOVA, O. A. (2002):** Hydrocyte system: structure and functions. – II Int. Conf. on plant anatomy and morphology; St.-Petersburg 14–18 October 2002. Abstract book: 21. [In Russian]
- BARYKINA, R. P., KOSTRIKOVA, L. N. & KOCHEMAROVA, I. P. (1971):** The practice of plant anatomy. – Moscow: Lomonosov Moscow State University. [In Russian]
- BARYKINA, R. P. & LOTOVA, L. I. (1962):** On the question of the vegetative propagation of *Pbeliodendron amurense*. – Vestnik Moskovskogo Universiteta. Seriya 6, biologiya **1**: 46–58. [In Russian]
- BARYKINA, R. P., VESELOVA, T. D. & DEVIYATOV, A. G. (2004):** Reference book on botanical micro-technique. The fundamentals and methods. – Moscow: Lomonosov Moscow State University. [In Russian]
- BOLWELL, G. P. (1989):** The use of tissue cultures for study of vessels differentiation. – In: DIXON, R. A. [ed.]: Plant cell culture: a practical approach: 128–153. – Oxford: IRL Press Limited.
- BUTENKO, R. G. (1964):** The culture of isolated tissues and the physiology of plant morphogenesis. – Moscow: Nauka. [In Russian]
- BUTENKO, R. G. (1975):** Differentiation and morphogenesis in tissue, cell and protoplast culture. – In: Plant developmental biology: 48–65. – Moscow: Nauka. [In Russian]
- CHURIKOVA, O. A. (1993):** Morphogenetic processes during the mass-clonal propagation of some ornamental bulbous and cormous plants. – Ph.D. Thesis, Lomonosov Moscow State University: Moscow. [In Russian]
- CHURIKOVA, O. A. & BARYKINA, R. P. (1995):** Regeneration capacity of some bulbous and cormous monocotyledons *in vitro*. The morphogenetic aspect. – Vestnik Moskovskogo Universiteta, Biologiya **2**: 58–66. [In Russian]
- CHURIKOVA, O. A., RUMYNIN, V. A., BARYKINA, R. P. & SLYUSARENKO, A. G. (1991):** Some peculiarities of the morphogenesis *in vitro* during the mass-clonal propagation of lilies. – Bulletin Glavnogo Botanicheskogo sada Akademii nauk **159**: 43–49. [In Russian]
- CHURIKOVA, O. A., RUMYNIN, V. A., BARYKINA, R. P. & SLYUSARENKO, A. G. (1994):** Morphogenetic processes in bulb scales of some lilies species during the mass-clonal propagation *in vitro*. – Bulletin Glavnogo Botanicheskogo sada Akademii nauk **169**: 105–111. [In Russian]
- ESAU, C. (1980):** Anatomija semennykh rastenij. Kn. 1. – Moscow: Izd. Mir. [Russian translation]
- GRIFFITH, M. M. (1957):** Foliar ontogeny in *Podocarpus macrophyllus*, with special reference to the trans-fusion tissue. – Amer. J. Bot. **44**: 705–715.
- HUBER, B. (1947):** Zur Microtopographie der Saftströme im Transfusionsgewebe der Koniferennadel. – Planta **35**: 331–351.
- KANIEWSKI, K. (1967):** Development of the hydrocyte (storage tracheid) system in the pericarp of *Raphanus sativus* L. – Bull. Acad. Pol. Sci. Biol. **15**(3): 171–180.
- KANIEWSKI, K. & WAŻYŃSKA, Z. (1968):** Development of pericarp in *Ornithopus sativus* L. – Bull. Acad. Pol. Sci. Biol. **16**(5): 303–306.
- LEDERER, B. (1955):** Vergleichende Untersuchungen über das Transfusionsgewebe einiger rezenter Gymnospermen. – Bot. Studien Heft **4**: 1–42.
- LOTOVA, L. I. & LYARSKAYA, R. P. (1959):** Some anatomical features of root accretion of *Cedrus deodara* and *C. atlantica*. – Nauchnyie doclady Vyshei Shkoly. Biologicheskiye nauki **4**: 99–104. [In Russian]

- LOTOVA, L. I., LYARSKAYA, R. P & YAROSLAVTSEV, G. D. (1960):** The anatomy of knitted up roots in two *Cedrus* species. – Sbornik rabot po dendrologii i decorativnomu sadovodstvu. Gosudarstvennyi Nikitskii botanicheskii sad: 81–99. [In Russian]
- LYARSKAYA, R. P. (1957):** The formation of adventitious buds on the *Malus* roots. – Vestnik Moscovskogo Universiteta 4: 53–62. [In Russian]
- PERVUCHINA, N. V. (1945):** Materials to the elucidating of callus formation peculiarities. – Soviet Botany 13(2): 51–60. [In Russian]
- RAUH, W. (1937):** Die Bildung von Hypokotyl- und Wurzelsprossen und ihre Bedeutung für die Wuchsformen der Pflanzen. – Nova Acta Leopoldina N.F 4(24): 395–553.
- ROBERTS, L. W. (1976):** Cytodifferentiation in plants. Xylogenesis as a model system. – Cambridge, New York, Melbourne: Cambridge University Press.
- RUMYNIN, V. A. & SLYUSARENKO, A. G. (1989):** The mass-clonal propagation of lilies. – Bulletin Glavnogo Botanicheskogo sada Akademii nauk 153: 62–69. [In Russian]
- YATSENKO-KHMELEVSKY, A. A. (1961):** The concise course of plant anatomy. – Moscow: Vysshaya shkola. [In Russian]

Address of the authors:

Dr. Olga A. Churikova
Prof. Dr. Rimma P Barykina
Higher Plants Department
Biological Faculty
Moscow State University
119992 Moscow
Russia
E-mail barykina@herba.msu.ru