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The plant tissue culture collection at the Department of Botany, University of Debrecen

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ABSTRACT We present a list of tissue culture systems developed at the Department of Botany, University of Debrecen. Several of tissue cultures were developed for the first time in our laboratory. These include micropropagation of *Elatine hungarica*, callus cultures of several oak genotypes of Hungarian origin and of *Crocus* species characteristic for the Carpathian Basin. Callus cultures were either organogenic (e.g. *Crocus scepusiensis*) or embryogenic (*Quercus petraea*, *Galanthus* and *Crocus* species, *Phragmites australis*). In case of embryogenic cultures, somatic embryos showed either normal, bipolar development (e.g. *Crocus heuffelianus*, *Phragmites australis*) or they have lost this bipolarity at the maturation of embryos (*C. banaticus*, *C. sativus*, *Galanthus*). The type of callus is yet to be identified for several cultures (e.g. *Plantago lanceolata*, *Vicia faba*). Most of our *in vitro* cultures proved to have plant regeneration potential. Several of them derived from endangered/ red list plants (*Crocus* species, *Elatine hungarica*, *Galanthus nivalis*), therefore they are suitable for germplasm preservation. Others (*Quercus*, *Phragmites australis*) proved to be suitable for stress physiology and/ or cell biology experiments. Cultures such as *Crocus sativus* or *Plantago lanceolata* derived from plants of medicinal importance, therefore are of potential pharmacological use.

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KEY WORDS

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Plant tissue culture procedures exploit the totipotency of plant cells and are widely used for different purposes. These include mainly biotechnological applications and by providing fully controllable systems, they are preferentially used in basic research, such as biochemistry, physiology and plant cell biology as well (Dodds and Roberts 1986; George et al. 2008). If genetic or epigenetic variability called somaclonal variation can be avoided, *in vitro* cultures can be used for the maintenance of agriculturally important or rare genotypes, being suitable for germplasm preservation (Heywood and Iriondo 2003). In the past years, we have developed a significant number of plant tissue cultures of real or potential use for the above purposes. These cultures are presented in brief in the forthcoming sections.

Materials and Methods

All plant *in vitro* cultures were established from explants of field-grown individuals, therefore subjected to surface sterilization in order to assure aseptic conditions. In general, this involved treatment with commercial bleach (8-10%, v/v) followed by several washes with sterile distilled water. Media

used were Murashige-Skoog basal medium with Gamborg's vitamins (Murashige and Skoog 1962; Gamborg et al. 1968) and 2% (w/v) sucrose (Reanal or Molar, Budapest, Hungary) or WPM medium (Woody Plant Medium, Lloyd and McCown 1980) solidified with 0.8% (w/v) agar (Difco, Lawrence, KS, USA). The nature of plant growth regulators (PGRs) depended on source explants and species. Auxins used were 2,4-dichlorophenoxyacetic acid (2,4-D), 3-indoleacetic acid (IAA), indole-3-butyric acid (IBA) and α -naphthaleneacetic acid (NAA) and the cytokinins were N⁶-benzyladenine (BA) or kinetin (KIN). All PGRs were from Sigma-Aldrich, Budapest, Hungary. In general, physical conditions were 14/10 (light/dark) photoperiod (cool white fluorescent lamps, 10-40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon fluence rate) with temperatures of 22/18 \pm 4°C. Callus cultures were subject of histological analysis in order to identify their type.

Results and Discussion

We have established tissue cultures from genotypes of 26 species belonging to Gymnosperms, Magnoliopsida and Liliopsida. These cultures are summarized on Table 1. Several of them were established in our laboratory for the first time, e.g. embryogenic cultures of *Crocus heuffelianus* (Demeter et al. 2010) or of Hungarian oak genotypes and a micropropagation

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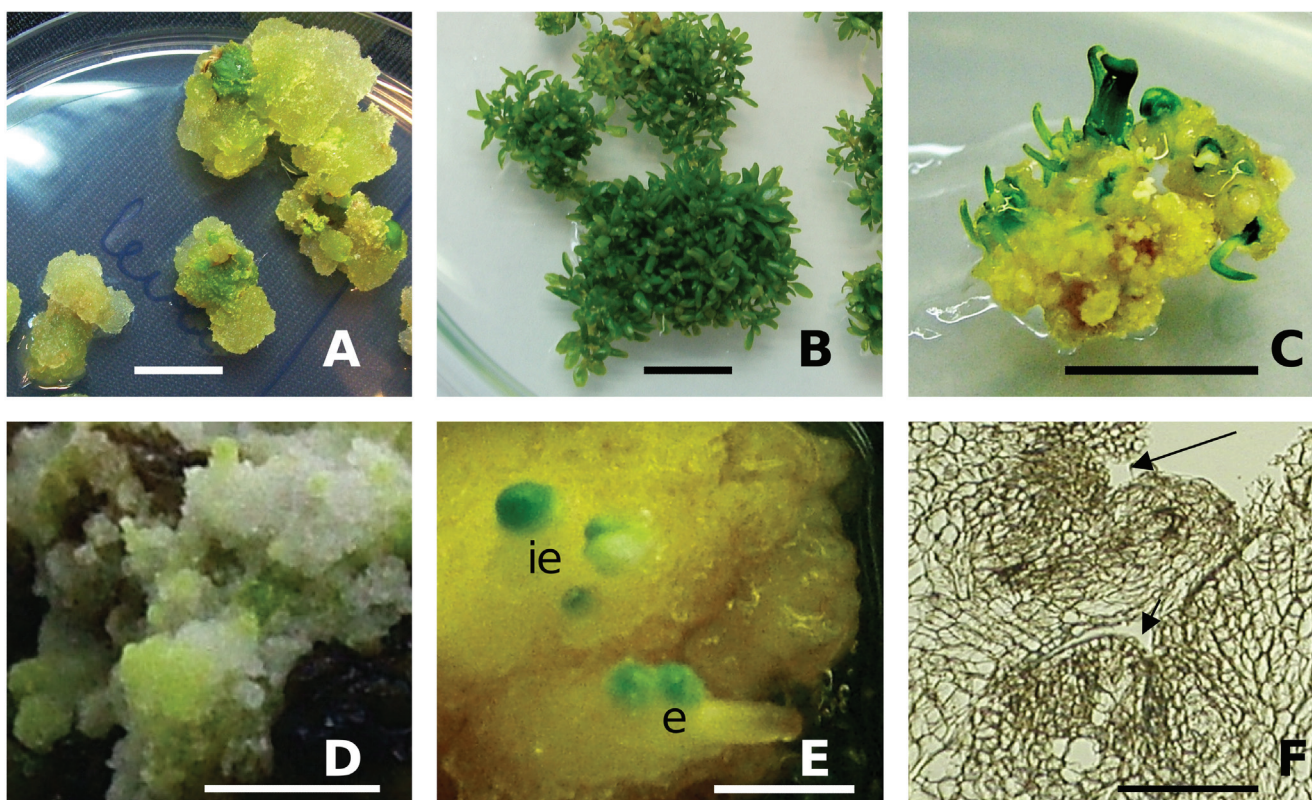


Figure 1. Examples of tissue cultures from the culture collection. A- leaf-derived calli of *Ginkgo biloba*; B- micropropagated shoots of *Elatine hungarica*; C- embryogenic callus of *Phragmites australis* from axillary buds of plants from Tihany, Lake Balaton. Coleoptiles and regenerated plantlets are seen; D- detail of an embryogenic callus of *Quercus petraea*; E- detail of an embryogenic callus of *Crocus heuffelianus* showing immature (ie) and mature (e) embryos; F- detail of an organogenic callus of *C. scepusiensis* showing shoot primordia (arrows). Scalebars: 10 mm (A-C), 2 mm (D, E), 200 μ m (F).

system of *Elatine hungarica* (Fig. 1 B, D, E). It should be noted that *Crocus* species of the Carpathian Basin- *C. banaticus*, *C. heuffelianus*, *C. scepusiensis*, *C. tommasianus* as well as *Drosera rotundifolia*, *Elatine hungarica* and *Galanthus nivalis*, are red list or potentially endangered species for the whole geographical region or in Hungary in particular- thus their cultures are important for germplasm preservation purposes. In case of *C. heuffelianus* we have already demonstrated that no somaclonal variation occurred during its long-term culture (Demeter et al. 2010) and its embryogenic cultures are able of regenerating whole plants with corms- this system is ready to be used for *ex vitro* cultivation. Other cultures proved to be useful in physiology/ cell biology experiments. For example, plants regenerated from embryogenic calli of common reed (*Phragmites australis*) proved to be useful in the elucidation of cellular effects of the protein phosphatase inhibitory cyanotoxin, microcystin-LR (see Máthé et al. 2007, 2009 for examples). Cultures like calli of *Ginkgo biloba* (Fig. 1A), *Crocus sativus*, *Plantago lanceolata*, *Solanum nigrum* or *Thymus vulgaris* are potentially useful for the production of pharmacologically important compounds, since they derived

from well-known medicinal plants (see Gonda et al. 2010, 2012 for examples).

For callus cultures, we could identify the callus type of several species/ genotypes. For *Quercus petraea*, most of *Crocus* species, *Galanthus* and *Phragmites australis*, we detected embryogenic calli. In case of *Q. petraea*, *C. heuffelianus* and *P. australis*, those embryos showed typical bipolar development and the latter two cultures were capable of efficient plant regeneration (Fig. 1C, E and Máthé et al. 2000; Demeter et al. 2010). In contrast, in case of *C. banaticus*, *C. sativus* and *Galanthus*, somatic embryos have lost their bipolarity: radicles failed to develop in mature embryos and root production during plant regeneration was a secondary process (data not shown). Although monopolar embryos are known for some tissue cultures (Blázquez et al. 2009), the cause of this type of development needs to be elucidated. Interestingly, calli of *C. scepusiensis* were organogenic and not embryogenic (Fig. 1F), even though the parent plants are believed to be genetically close related to *C. heuffelianus* (Rafinski and Passakas 1976) that produced embryogenic calli.

Table 1. The tissue culture collection of the Department of Botany, University of Debrecen.

Species	Cultivar/ genotype/ culture line	Location of source plants	Explant type	Culture type	References for culture conditions	Uses
Gymnosperms						
1. <i>Ginkgo biloba</i>	n.d.	Botanical Garden, UD, Hungary	young leaves, buds	callus (undefined type)	-	germplasm preserva- tion, potential phar- macological use
Magnoliopsida						
2. <i>Drosera rotundi- folia</i>	n.d.	n.d.	whole plants	micropropagated plants	-	germplasm preserva- tion
3. <i>Elatine hungarica</i>	n.d.	Konyár, NE Hun- gary	whole plants	micropropagated plants	-	germplasm preserva- tion
4. <i>Quercus robur</i>	n.d.	Sikfőkút, NE Hun- gary	winter buds	embryogenic callus	-	germplasm preserva- tion
5. <i>Quercus petraea</i>	A149, A71	Sikfőkút, NE Hun- gary	winter buds, catkins	embryogenic callus	-	germplasm preser- vation, physiology experiments
6. <i>Q. polycarpa</i>	C211	Sikfőkút, NE Hun- gary	winter buds, catkins	callus (undefined type), capable of organogenesis	-	germplasm preserva- tion
7. <i>Q. pubescens</i>	A75	Sikfőkút, NE Hun- gary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preser- vation, physiology experiments
8. <i>Q. virgiliana</i>	B50	Sikfőkút, NE Hun- gary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preser- vation, physiology experiments
9. <i>Q. petraea</i> x <i>Q. dalechampii</i>	D137	Sikfőkút, NE Hun- gary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preser- vation, physiology experiments
10. <i>Q. petraea</i> x <i>Q. polycarpa</i>	C102, K28	Sikfőkút, NE Hun- gary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preserva- tion
11. <i>Q. petraea</i> x <i>Q. pubescens</i>	D89	Sikfőkút, NE Hun- gary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preser- vation, physiology experiments
12. <i>Q. virgiliana</i> x <i>Q. polycarpa</i>	A211	Sikfőkút, NE Hun- gary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preser- vation, physiology experiments
13. <i>Plantago lan- ceolata</i>	n.d.	Hajdúsámson, NE Hungary	young leaves, roots	organogenic callus	-	potential pharmaco- logical use
14. <i>Sinapis alba</i>	Budakalá- szi sárga	commercially avail- able	hypocotyls	callus (undefined type)	-	physiology experi- ments
15. <i>Solanum nigrum</i>	n.d.	Debrecen, Hungary	young leaves	callus (undefined type)	-	potential pharmaco- logical use
16. <i>Thymus vulgaris</i>	n.d.	Debrecen, Hungary	shoots	callus (undefined type) capable of shoot regenera- tion	-	potential pharmaco- logical use
17. <i>Vicia faba</i>	ARC Egypt cross	Egypt, commercial source	shoots, young leaves	callus (undefined type) capable of shoot regenera- tion	Molnár, 1993	physiology experi- ments
Liliopsida						
18. <i>Crocus banaticus</i>	n.d.	Finis, Sovata (Ro- mania)	corms	embryogenic callus, capable of plant regeneration	-	germplasm preserva- tion
19. <i>C. heuffelianus</i>	n.d.	Rodnei mts, Romania; Zakar- patska obl. County (Ukraine)	seeds, corms	embryogenic callus, capable of plant regeneration	Demeter et al., 2010	germplasm preserva- tion
20. <i>C. sativus</i>	n.d.	Budapest, Hungary (from private gar- den)	corms	embryogenic callus, capable of plant regeneration	-	germplasm preserva- tion, potential phar- macological use
21. <i>C. scepusiensis</i>	n.d.	Kriva, Slovakia	seeds	organogenic callus, capable of plant regeneration	-	germplasm preserva- tion

Table 1. Continued.

22. <i>C. tommasinianus</i>	n.d.	Gyulaj, Hungary	seeds	callus (undefined type)	-	germplasm preservation
23. <i>Galanthus nivalis</i>	A	Hollóháza, Hungary	bulb scales	embryogenic callus, capable of plant regeneration	-	germplasm preservation, potential pharmacological use
	B, C	Tokaj, Hungary	bulb scales	embryogenic callus, capable of plant regeneration; shoot micropropagation, without callus stage	-	germplasm preservation, potential pharmacological use
24. <i>G. elwesii</i>	n.d.	The Netherlands, commercial source	bulb scales	embryogenic callus, capable of plant regeneration	-	germplasm preservation, potential pharmacological use
25. <i>G. woronowii</i>	n.d.	The Netherlands, commercial source	bulb scales	embryogenic callus, capable of plant regeneration	-	germplasm preservation, potential pharmacological use
26. <i>Phragmites australis</i>	1	Botanical Garden, UD, Hungary	Stem nodes, roots	embryogenic callus	Máthé et al., 2000, 2012	physiology experiments
	2	Debrecen-Józsa, Hungary	Stem nodes, roots	embryogenic callus, capable of plant regeneration if derived from stem nodes	Máthé et al., 2000, 2012	physiology experiments
	3	Tihany, Lake Balaton, Hungary	Stem nodes, axillary buds, roots	embryogenic callus, capable of plant regeneration if derived from stem nodes	Máthé et al., 2012	physiology experiments
	4	Kis-Balaton Reservoir, Hungary	Stem nodes, roots	embryogenic callus	Máthé et al., 2012	physiology experiments

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