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ARTICLE

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. **Acta Biol Szeged 56(2):179-182 (2012)**

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 N^6-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 μmol m^{-2} s^{-1} photon fluence rate) with temperatures of 22/18 ± 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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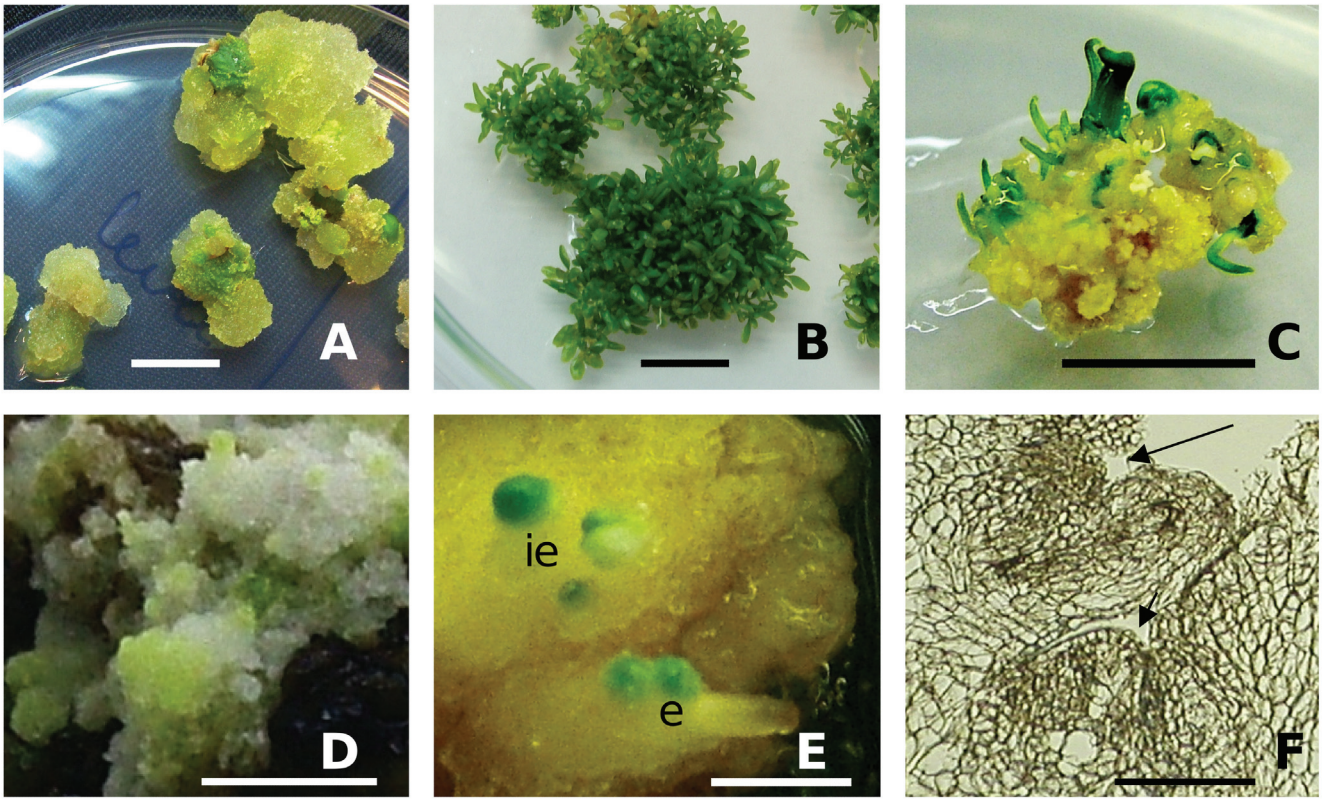

Figure 1. Examples of tissue cultures from the culture collection. A- leaf-derived calli of *Ginkgo biloba*; B- micropropagated shoots of *Elatine hungarica*; C- embryogenic callus of *Phragmites australis* from axillary buds of plants from Tihany, Lake Balaton. Coleoptiles and regenerated plantlets 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 µm (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. tommasinianus* 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. *Ginkgo biloba*	n.d.	Botanical Garden, UD, Hungary	young leaves, buds	callus (undefined type)	-	germplasm preservation, potential pharmacological use
Magnoliopsida						
2. *Drosera rotundifolia*	n.d.	n.d.	whole plants	micropropagated plants	-	germplasm preservation
3. *Elatine hungarica*	n.d.	Konyár, NE Hungary	whole plants	micropropagated plants	-	germplasm preservation
4. *Quercus robur*	n.d.	Síkfőkút, NE Hungary	winter buds	embryogenic callus	-	germplasm preservation
5. *Quercus petraea*	A149, A71	Síkfőkút, NE Hungary	winter buds, catkins	embryogenic callus	-	germplasm preservation, physiology experiments
6. *Q. polycarpa*	C211	Síkfőkút, NE Hungary	winter buds, catkins	callus (undefined type), capable of organogenesis	-	germplasm preservation
7. *Q. pubescens*	A75	Síkfőkút, NE Hungary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preservation, physiology experiments
8. *Q. virgiliana*	B50	Síkfőkút, NE Hungary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preservation, physiology experiments
9. *Q. petraea* x *Q. dalechampii*	D137	Síkfőkút, NE Hungary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preservation, physiology experiments
10. *Q. petraea* x *Q. polycarpa*	C102, K28	Síkfőkút, NE Hungary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preservation
11. *Q. petraea* x *Q. pubescens*	D89	Síkfőkút, NE Hungary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preservation, physiology experiments
12. *Q. virgiliana* x *Q. polycarpa*	A211	Síkfőkút, NE Hungary	winter buds	callus (undefined type), capable of organogenesis	-	germplasm preservation, physiology experiments
13. *Plantago lanceolata*	n.d.	Hajdúsámson, NE Hungary	young leaves, roots	organogenic callus	-	potential pharmacological use
14. *Sinapis alba*	Budakalászi sárga	commercially available	hypocotyls	callus (undefined type)	-	physiology experiments
15.*Solanum nigrum*	n.d.	Debrecen, Hungary	young leaves	callus (undefined type)	-	potential pharmacological use
16. *Thymus vulgaris*	n.d.	Debrecen, Hungary	shoots	callus (undefined type) capable of shoot regeneration	-	potential pharmacological use
17. *Vicia faba*	ARC Egypt cross	Egypt, commercial source	shoots, young leaves	callus (undefined type) capable of shoot regeneration	Molnár, 1993	physiology experiments
Liliopsida						
18. *Crocus banaticus*	n.d.	Finis, Sovata (Romania)	corms	embryogenic callus, capable of plant regeneration	-	germplasm preservation
19. *C. heuffelianus*	n.d.	Rodnei mts, Romania; Zakarpatska obl. County (Ukraine)	seeds, corms	embryogenic callus, capable of plant regeneration	Demeter et al., 2010	germplasm preservation
20. *C. sativus*	n.d.	Budapest, Hungary (from private garden)	corms	embryogenic callus, capable of plant regeneration	-	germplasm preservation, potential pharmacological use
21. *C. scepusiensis*	n.d.	Kriva, Slovakia	seeds	organogenic callus, capable of plant regeneration	-	germplasm preservation

Table 1. Continued.

22. *C. tommasinianus*	n.d.	Gyulaj, Hungary	seeds	callus (undefined type)	-	germplasm preservation
23. *Galanthus nivalis*	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. *G. elwesii*	n.d.	The Netherlands, commercial source	bulb scales	embryogenic callus, capable of plant regeneration	-	germplasm preservation, potential pharmacologi-cal use
25. *G. woronowii*	n.d.	The Netherlands, commercial source	bulb scales	embryogenic callus, capable of plant regeneration	-	germplasm preservation, potential pharmacologi-cal use
26. *Phragmites australis*	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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References

Blázquez S, Olmos E, Hernández JA, Fernández-Garcia N, Fernandez JA, Piqueras A (2009) Somatic embryogenesis in saffron (*Crocus sativus* L.). Histological differentiation and implication of some components of the antioxidant enzymatic system. Plant Cell Tiss Org 97:49-57

Demeter Z, Surányi G, Molnár VA, Sramkó G, Beyer D, Kónya Z, Vasas G, M-Hamvas M, Máthé C (2010) Somatic embryogenesis and regeneration from shoot primordia of *Crocus heuffelianus*. Plant Cell Tiss Org 100:349-353.

Dodds JH, Roberts LW (1986) Experiments in plant tissue culture. Cambridge University Press, Cambridge- London- New York- New Rochelle-Melbourne- Sydney.

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151-158.

George EF, Hall MA, De Klerk G-J, Eds. (2008) Plant propagation by tissue culture, vol. 1.:The background. Springer, Dordrecht.

Gonda S, Tóth L, Parizsa P, Nyitrai M, Vasas G (2010) Screening of common *Plantago* species in Hungary for bioactive molecules and antioxidant activity. Acta Biol Hung 61:25-34.

Gonda S, Parizsa P, Surányi G, Gyémánt G, Vasas G (2012) Quantification of main bioactive metabolites from saffron (*Crocus sativus*) stigmas by a micellar electrokinetic chromatographic (MEKC) method. J Pharmaceut Biomed 66:68-74.

Heywood VH, Iriondo JM (2003) Plant conservation: old problems, new perspectives. Biol Conserv 113:321-335.

Lloyd GB, McCown BH (1980) Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. Proc Int Plant Propagators Soc 30:421-37.

Máthé C, M. Hamvas M, Grigorszky I, Vasas G, Molnár E, Power J B, Davey M R, Borbély G (2000) Plant regeneration from embryogenic calli of *Phragmites australis* (Cav.) Trin. Ex Steud. (common reed). Plant Cell Tiss Org 63:81-84.

Máthé C, M-Hamvas M, Vasas G, Surányi G, Bácsi I, Beyer D, Tóth S, Tímár M, Borbély G (2007) Microcystin-LR, a cyanobacterial toxin, induces growth inhibition and histological alterations in common reed (*Phragmites australis* /Cav./ Trin. Ex Steud.) plants regenerated from embryogenic calli. New Phytol 176:824-835 .

Máthé C, Beyer D, Erdődi F, Serfőző Z, Székvölgyi L, Vasas G, M-Hamvas M, Jámbrik K, Gonda S, Kiss A, M. Szigeti Z, Surányi G (2009) Microcystin-LR induces abnormal root development by altering microtubule organization in tissue-cultured common reed (*Phragmites australis*) plantlets. Aquat Toxicol 92:122-130.

Máthé C, Mosolygó A, Surányi G, Beke A, Demeter Z, Tóth VR, Beyer D, Mészáros I, M-Hamvas M (2012) Genotype and explant-type dependent morphogenesis and silicon response of common reed (*Phragmites australis*) tissue cultures. Aquat Bot 97:57-63.

Molnár Z (1993) Az *in vitro* mikroszaporítási módszerek jelentősége a lóbab (*Vicia faba* L.) és borsó (*Pisum sativum* L.) nemesítésében. Acta Agr Óváriensis 35:119-123 (in Hungarian).

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plantarum 15:473-497.

Rafinski JN, Passakas T (1976) Chromosomal differentiation within *Crocus vernus* agg. (*Iridaceae*) in the Carpathian Mts. Plant Syst Evol 125:1-8.