GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITE DE BEOGRAD
Tom XVII, 1983, Beograd

UDK 581.4 : 576.72 : 582.657 (497.1)

VEROSLAVA SREJOVIĆ and MIRJANA NEŠKOVIĆ

FORMATION AND ANATOMY OF BUCKWHEAT ORGANOGENIC CALLUS TISSUE IN VITRO

Institute of Biology, Faculty of Science, Kragujevac
Institute of Botany, Faculty of Science, Beograd

Srejović, V. and Nešković, M. (1983): *Formation and anatomy of buckwheat organogenic callus tissue in vitro.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XVII, 45–50.

Isolated buckwheat cotyledons can be induced to dedifferentiate and produce a callus tissue, in which the meristemoids give rise to either roots, or shoots. A high 2,4–D content is optimal for dedifferentiation, while roots appear when its concentration is lowered. Buds are induced at high cytokinin (BAP) and low auxin (IAA) levels. Callus anatomy has been observed in the course of organ initiation and development. It has been demonstrated that after 2 weeks in culture the whole interior of leaf lamina is changed and transformed into numerous meristematic nodules. They consist of larger, vacuolated cells in the center and smaller, densely stained cells on the perifery. After decreasing 2,4–D content, some of the periferal cells divide more intensively and organize as root meristems. During the later subcultures, the entire callus tissue is gradually transformed into root initials; when the roots elongate, the tissue becomes necrotic. These cultures stop growing after 5–6 passages. In contrast, tissue grown on bud induction medium contains meristemoids which soon give rise to typical shoot apical meristems. In this case the callus is capable of permanent proliferation and it constantly produces numerous bud initials. Xylogenesis is also frequently observed in that tissue.

Key words: root development, shoot development, callus anatomy, cytokinin/auxin ratio, *Fagopyrum esculentum* M o e n c h.
Ključne reči: razviće korena, razviće izdanka, anatomija kalusa, odnos citokinin/auksin, *Fagopyrum esculentum* M o e n c h.

INTRODUCTION

The capacity of isolated leaves and cotyledons to develop organogenic callus tissue *in vitro* has been demonstrated in many plant species so far. As in all plant explants, callus tissue was usually initiated by the action of auxins and cytokinins, although different plant species may have specific requirements in respect to the kind and ratio of these hormones in the medium. It has been reported in the previous paper (S r e j o v i ć and N e š k o v i ć, 1981) that isolated buckwheat *(Fagopyrum esculentum* M o e n c h.) cotyledons have the capacity to produce callus tissue, in which organs and whole plants can be regenerated. Sequential changes of the hormones in the medium were essential for the formation of either roots, or shoots in the callus. The present paper describes the anatomy of callus tissue in the course of root and shoot initiation. It has been demonstrated that meristemoids, which develop in the initial leaf explants, can be determined at an early stage towards roots or shoots by specific hormonal composition of the medium.

MATERIAL AND METHODS

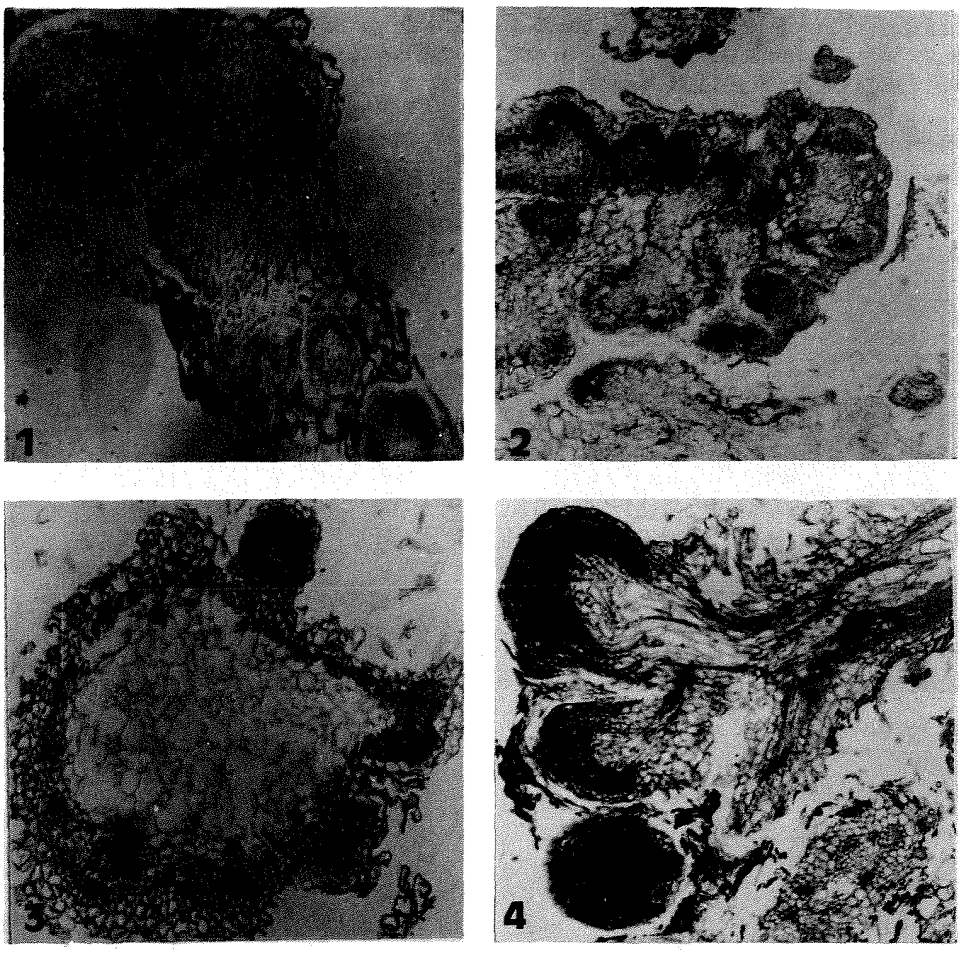
Cotyledons of buckwheat *(Fagopyrum esculentum* M o e n c h.), of the tetraploid variety Pennquad, were isolated from imbibed seeds as described previously (S r e j o - v i ć and N e š k o v i ć, 1981). They were cut transversely into two pieces and cultivated in a nutrient medium, which contained mineral solution B_5 (G a m b o r g *et al.*, 1968), 3% sucrose, 1% agar and (in mg l^{-1}): thiamin 10, pyridoxin 1, nicotinic acid 1, m–inositol 100 and casein hydrolysate 2000. Auxins 2,4–D (2,4–dichlorophenoxy-acetic acid) and IAA (indolyl–3–acetic acid) and cytokinins kinetin and BAP (6–benzylaminopurine) were added in different concentrations, as indicated in the text. Growth conditions were as previously described (S r e j o v i ć and N e š k o v i ć, 1981).

Pieces of callus tissue were fixed in Carnoy fixative, embedded in paraffin, and sections of 10 μm were stained with safranin and light green.

RESULTS

Cotyledon fragments were initially put on a medium containing 2,4–D 5 mg l^{-1} and kinetin 0.1 mg l^{-1}. During the first 5 days the surface of the explants enlarged 2–3 times and the lamina acquired normal green colour. As already reported, changes in the anatomy of cotyledon fragments could be observed within 3–5 days in culture. In the cotyledons, having the structure of a typical leaf, mesophyll cells between the vascular bundles and around the main leaf vein start dividing. Cell divisions proceed soon in other mesophyll cells and after 2 weeks the whole interior of the leaf was transformed and contained numerous meristematic nodules (Fig. 1). After 2–4 weeks a callus tissue developed, particularly on the cut surfaces of the leaf. The callus was nodulated, pale in colour, but comprised also red regions, due to the presence of anthocyanin. The callus was composed of large, vacuolated cells, while small cells with dense cytoplasm formed a rather uniform layer near the surface (Fig. 2).

If the explants remained in the initial medium with high 2,4–D content, the callus rather quickly deteriorated and became necrotic. However, if the explants were transferred within 2–4 weeks to the medium containing 0.1 or 1.0 mg l^{-1} 2,4–D, a highly



Figs. 1–4. – Root development in cotyledon explants and callus.

Fig. 1. – Meristematic nodules in cotyledon explant after 2 weeks in culture on 2,4–D (5 mg l^{-1}) and kinetin (0.1 mg l^{-1}), 70 x.

Fig. 2. – Section of callus tissue after 30 days in culture, 50 x.

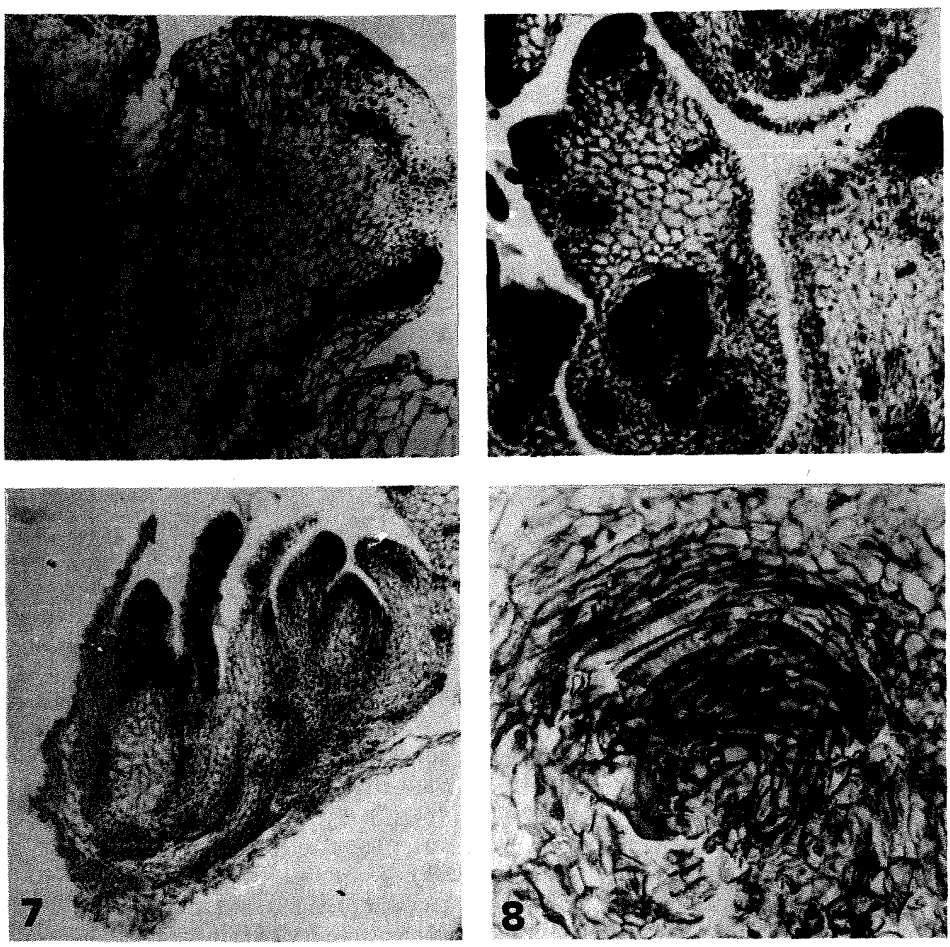
Fig. 3. – Section through a small callus nodule during the first subculture, transferred to 2,4–D 1 mg l^{-1}; note the periferal meristem and localized root initials, 30 x.

Fig. 4. – Section through the callus tissue during the 5th subculture; note numerous root initials and the absence of parenchymatous tissue between them, 30 x.

nodulated callus developed during the subsequent subcultures. Microscopic examination of a small nodule, fixed during the first subculture, reveals parenchymatous cells in the center, surrounded by a continuous layer of rectangular, deeply stained meristematic cells. These cells in some parts divide more intensively, to form root primordia (Fig. 3). A nodule fixed in the fifth subculture consisted mainly of root primordia, or some elongated roots, with very little parenchymatous tissue between them (Fig. 4). During the

passages on media with decreased amount of 2,4–D, numerous root initials develop into roots, but this eventually leads to the death of the callus. It was not possible to maintain that callus longer than for 5–6 passages.

Development of buds could be initiated only if the cotyledon explants were removed from the high 2,4–D medium after 3–5 days, and cultivated further on a medium supplemented by 10^{-5} M BAP and 10^{-6} M IAA. On this medium a callus tissue



Figs. 5–8. – Bud development in callus tissue.

Fig. 5. – Section through a callus tissue after 45 days on BAP (10^{-5} M) and IAA (10^{-6} M) medium, showing bud primordia and meristematic centers, 90 x.

Fig. 6. – Section through a caulogenic callus at the end of 8th subculture; note numerous bud initials, 65 x.

Fig. 7. – Leafy bud initials at the end of 8th subculture, 37 x.

Fig. 8. – Vascular nodule formed during the 2nd subculture on BAP (10^{-5} M) and IAA (10^{-6} M) medium, 120 x.

was also obtained. Observation by the end of the first subculture or during the second one, showed that callus proliferations were composed of large vacuolated cells and meristematic zones in the periferal parts, which represent bud primordia. Small scattered groups of meristematic cells are seen also deeper in the parenchymatous tissue (Fig. 5). The callus retains similar structure during many later subcultures (Fig. 6) and bud primordia constantly develop into normal buds (Fig. 7). The capacity for budding of that callus was persistent for more than 3 years.

It is characteristic for the caulogenic callus, that meristemoids inside the tissue frequently develop into vascular nodules, with concentric layers of tracheidal cells (Fig. 8).

DISCUSSION

The understanding of root and shoot development in callus cultures is mainly based on two concepts, put forward about 20 years ago. Skoog and Miller (1957) demonstrated that the balance between auxin and cytokinin in the medium is most important in determining whether root or shoot primordia will be organized. Although in the years that followed some exceptions were reported, this balance has been widely recognized as the main factor of organ determination. A few years later, Steward *et al.* (1967) pointed out to the importance of sequential changes in hormone complement, as to the possible means to induce organogenesis in some recalcitrant tissues. It has become clear that the whole process of organogenesis consists of separate phases, each requiring a specific hormonal composition and balance (Söndahl and Sharp, 1977; Walker *et al.*, 1979).

The results obtained with buckwheat tissue can be considered as a support to both concepts. Callus tissue can be induced in cotyledon fragments in all media used, but organ formation depends on the ratio of auxins and cytokinins. The structure of the callus tissue is in the beginning typical (see Gautheret, 1959), and consists of a mass of parenchymatous cells with meristematic centers, responsible for callus growth. The inductive hormone combinations switch the development of these meristemoids into root or shoot primordia.

The second concept, concerning the necessity of sequential hormone changes, is illustrated by the development of organ initials. The best conditions for cell dedifferentiation and division (high 2,4–D content) are not suited for organ induction. Auxin concentration has to be lowered to permit root growth, while both ratio and kind of hormones have to be altered, in order to obtain abundant bud development. Thus, in the first phase cells are stimulated to divide and produce meristematic centers, which for some time remain capable for dual role. The first phase may not be indispensible for organogenesis, however. Buds can be induced when the cotyledon explants are put directly on the inductive medium, but the frequency of organogenesis and the number of buds in that case is much reduced, in comparison to the explants grown initially on 2,4–D (results in preparation). A sequence of the media is, therefore, required for the expression of full regeneration potential of callus tissue.

Microscopic examination of calluses grown in the two media, reveals differences between them. Although 2,4–D induces high rate of cell proliferation, true callus tissue is apparently not obtained. It seems that the „tissue" largely consists of root initials; when they elongate, there is little tissue left to be subcultured. Similar structure was described for maize tissue culture (Mott and Cure, 1978). On the contrary, callus developed

on BAP – IAA medium has a potential for permanent growth, although it also produces organs constantly. Formation of vascular nodules inside that callus is probably due to the high cytokinin content in the medium (B e r g m a n n, 1964).

REFERENCES

B e r g m a n n, L. (1964): Der Einfluss von Kinetin auf die Ligninbildung und Differenzierung in Gewebekulturen von *Nicotiana tabacum.* – Planta, *62:* 221–254.

G a m b o r g, O. L., M i l l e r, A., O j i m a, K. (1968): Nutrient requirements of suspension cultures of soybean root cells. – Exp. Cell Res., *50:* 152–158.

G a u t h e r e t, R. G. (1959): La culture des tissues végétaux. – Masson, Paris.

M o t t, R. L., C u r e, W. W. (1978): Anatomy of maize tissue cultures. – Physiol. Plant., *42:* 139–145.

S k o o g, F., M i l l e r, C. O. (1957): Chemical regulation of growth and organ formation in plant tissues cultured *in vitro.* – Symp. Soc. Exptl. Biol., *11:* 118–131.

S o n d a h l, M. R., S h a r p, W. R. (1977): High frequency of somatic embryos in cultured leaf explants of *Coffea arabica* L. – Z. Pflanzenphysiol., *81:* 395–408.

S r e j o v i ć, V., N e š k o v i ć, M. (1981): Regeneration of plants from cotyledon fragments of buckwheat *(Fagopyrum esculentum* M o e n c h.). – Z. Pflanzenphysiol., *104:* 37–42.

S t e w a r d, F. C., K e n t, A. E., M a p e s, M. O. (1967): Growth and organization in cultured cells: Sequential and synergistic effects of growth regulating substances. – Ann. N.Y. Acad. Sci., *144:* 326–334.

W a l k e r, K. A., W e n d e l n, M. L., J a w o r s k i, E. G. (1979): Organogenesis in callus tussue of *Medicago sativa.* The temporal separation of induction processes from differentiation processes. – Plant Sci. Letters, *16:* 23–30.

R e z i m e

VEROSLAVA SREJOVIĆ i MIRJANA NEŠKOVIĆ

OBRAZOVANJE I ANATOMIJA ORGANOGENOG KALUSNOG TKIVA HELJDE IN VITRO

Institut za biologiju, Prirodno–matematički fakultet, Kragujevac
Institut za botaniku, Prirodno–matematički fakultet, Beograd

Izolovani kotiledoni heljde *(Fagopyrum esculentum)* proizvode u kulturi kalusno tkivo sa visokom organogenom sposobnošću. Proučavana je anatomija kalusa u toku inicijacije i razvića korenova, odnosno izdanaka. Utvrđeno je da su dediferencirane meristemske ćelije sposobne za dvojak razvoj, ali da postaju determinisane za razviće u koren ili u pupoljak pod dejstvom adekvatnog odnosa citokinin/auksin.