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SPECIFICITY OF CYTODIFFERENTIATION IN CALLUSES IN VITRO OF *AESCULUS HIPPOCASTANUM* FORM RESISTANT TO HORSE-CHESTNUT LEAF MINER

A.F. LIKHANOV[1], O.V. OVERCHENKO[2], S.M. KOSTENKO[2], O.V. SUBIN[2]

[1]Institute for Evolutionary Ecology, National Academy of Sciences of Ukraine
37 Acad. Lebedeva St., Kyiv, 03143, Ukraine
[2]National University of Life and Environmental Sciences of Ukraine
15 Heroiv Oborony St., Kyiv, 03041, Ukraine

This paper present researches of callusogenesis specificity and cell differentiation of resistant (RP) and non-resistant (NRP) forms to horse-chestnut leaf miner (HCLM) of *Aesculus hippocastanum* according peculiarities of plant tissue primary and secondary metabolism. The most active callusogenesis was observed on DKW medium, with the addition of 0.5 mg/L kinetin and 3.0 mg/L 2,4-D. Structurally, three areas in calluses of RP and NRP forms could be recognized. The superficial part (area III) of callus in RP form was formed by cells filled with condensed tannins, with thick cell walls. A layer of parenchyma with thin cell walls underlined the outer layer of callus (area II). Numerous tracheate elements, capable of accelerated transportation of nutrients into tissues supporting cellular nutrition and differentiation, were formed among them. The internal area (area I) consisted of parenchymal cells. Many of them had in protoplasts the amorphous structures with polysaccharide and tannin complexes. The lignification of the parenchyma cell walls in callus tissues occurred under increasing of anionic peroxidases activity. This rate was five or more times higher than for the NRP form. Concerning to the RP form of *Aesculus hippocastanum* the viscosity of leaf cell juice may be the key factor limiting HCLM larval development. The amount of phenols in the leaves is not related with it. The kinematic viscosity of the RP form leaves (1,889 mm^2/s) was 1.53 times higher than that of NRP form (1.214 mm^2/s). In contrast, the content of phenolic substances was twice higher in the NRP form. The confirmed metabolic specificity of RP form can be explained by the relatively richer quantitative and qualitative composition of free amino acids in its tissues, compared to the NRP form. In general, the metabolism specificity of RP form callus tissues is a convenient model for studying the mechanisms of resistance against pathogens and pests of common horse chestnut.

Key words: *Aesculus hippocastanum* L., common horse chestnut, callus, cells, regeneration, morphogenesis.

Recently in Ukraine, the stands of common horse chestnut (*Aesculus hippocastanum* L.) have been seriously damaged by the invasive horse-chestnut leaf miner (HCLM) *Cameraria ohridella* Deschka & Dimic. The larvae of massively reproducing HCLM can significantly reduce the trees' phytosanitary functions, general condition and decorative value by the middle of the vegetative season [3]. Finding plants with pronounced antifeedant properties and increased resistance to the phytophage to use them as donors for microclonal propagation is one of the approaches to solving this problem. Micropropagation of *Aesculus hippocastanum* L. has been successful. For example, Gas-

taldo et al. [11] used the stem explants of *Aesculus hippocastanum* cultivated on a solid agar Murashige and Skoog (MS) nutrient medium with 9.3 μM of kinetin, 0.7 μM of naphthaleneacetic acid (NAA) and 9.0 μM of 2,4-dichlorophenoxyacetic acid (2.4-D) for somatic embryogenesis *in vitro*. Chechenieva et al. [8] used the horse chestnut leaf blade tissues in cultivation *in vitro*. Alternatively, Radojevic [17] observed the somatic embryogenesis in the callus cultures obtained from the immature embryos of *Aesculus hippocastanum* seeds. The culture was grown on a modified MS medium with 2,4-D (3 mg/L), kinetin (1 mg/L), CH (250 mg/L) and proline (250 mg/L) to promote the embryogenesis processes. In the same study, reducing the 2,4-D content in solid MS to 1 mg/L was shown to accelerate cell proliferation in the calluses and increase the total number of somatic embryos. According to Sediva [20] the regeneration of tissues and organs of horse chestnut *in vitro* can be induced in different explants by changing the nutrient composition and concentration of growth regulators.

In Sediva's experiments, the strongest formation of adventive shoots from leaf segments in the WPM medium was induced by BA (benzyladenine) in 4.4—8.9 μM concentration range. The organogenesis in calluses was inhibited by adding 0.5 μM NAA to medium. At the same time, 2.7 μM NAA showed the best rhizogenic effect [20]. Profumo et al. [15] used the MS medium supplemented with 2.0 mg/L kinetin, 2.0 mg/L naphthyl acetic acid and 2.0 mg/L 2.4-D for the induction of embryogenesis in *Aesculus hippocastanum*. The induction was also stable under this hormonal profile in further experiments on the synthesis of escin [16]. Saito [19] used 10 mg/L IBA (3-indolebutyric acid), 0.06 mg/L NAA and 0.4 mg/L BAP, as well as *L*-cysteine, *L*-serine, *L*-glycine amino acids (1 mg/L) to induce callusogenesis. A short-term (4—5 days) increase of BAP concentration to 44 μM effectively stimulated the mass formation of somatic embryos [13]. Thus, there is still no unambiguous idea about the optimal balance of synthetic growth regulators in the medium composition. To a certain extent it is determined by the different mesophilic cell reactions to hormonal stimuli, which may occur even within one metamer. Welander [21] and Sarwar [18] reported that the regenerative capability of the leaf segments increases from the apical to the basal parts of the leaf blades, where cells appeared to be more sensitive to BAP and TDZ.

Thus, the physiological and biochemical specifics should be taken into account in *in vitro* cultivating and obtaining the regenerant plants of common horse chestnuts resistant forms. Hence, this work aimed to determine the composition of the nutrient medium to induce callusogenesis in HCLM-resistant horse chestnut form and to identify the histochemical and physiological characteristics in the tissues to optimize the conditions for obtaining regenerant plants and somatic embryogenesis.

Materials and methods

The calluses were taken from the plants of the common horse chestnut in Kyiv parks. The HCLM-resistant form of common horse chestnut was selected in accordance with the results of long-term monitoring studies (2010—2015).

The leaf blade fragments of the common horse chestnut forms resistant (RP) and non-resistant (NRP) to HLCM were used for the callus cultures. The explants were aseptically sterilized. The leaf blades were treated with 70 % C_2H_5OH for 30 s, then with 0.1 % $HgCl_2$ solution for 5 min and then trice washed with sterile distilled water for 10 min. For the cultivars, the leaf discs

of 12 mm in diameter were placed with adaxial surfaces facing the nutrient medium to obtain the callus cultures. Modified MS, DKW and WPM media were used to initiate the callusogenesis. The leaf blade fragments were kept in the dark for the first two weeks, and then were transferred to light.

The Cellulose HPTLC plates (Merck) with the chloroform-*n*-butanol/acetic acid/methanol/water (3/30/10/23 v/v/v/v) solvent system was used for chromatographic separation of free amino acids in the tissues of leaves and calluses. A solution of 0.3 % ninhydrin in *n*-butanol was used to identify the amino acids on the chromatogram. The 1 mg/ml amino acid solutions were used as standards.

In plant tissues, the activity of free and loosely-bound with cell walls peroxidases (PO) was determined according to Boyarkin [5, 6].

Anatomical and histochemical studies of the NRP and RP forms of *Aesculus hippocastanum* calluses were carried out on microtome cuts (8 μm). The plant tissues were stained with differential saphranine-water blue [7] and examined under Nikon Eclipse E-200 microscope (Japan).

Periodic acid-Schiff (PAS) staining system (Sigma Eldrich, pr. No. 395) was used for the detection of polysaccharides by the PS reaction. The qualitative composition of polysaccharides in callus cells was determined during their enzymatic hydrolysis by pectinase and hemicellulase. The dewaxed microtome sections (8 μm) of the callus tissues were treated in a humid chamber with 1.0 μM pectinase solution (Sigma Eldrich) in citrate buffer pH 4.0, 25 °C, for 20 min. The cuts were then trice washed with distilled water and the polysaccharide residues were detected by the PS reaction. The cuts were treated with 1.0 μM hemicellulase solution (Sigma Eldrich) in citrate buffer, pH 4.5, 40 °C for 5 min. The polysaccharides were detected by standard protocol (Sigma Eldrich, pr. No. 395) after washing with water.

The kinematic viscosity of the horse chestnut leaf aqueous extracts was determined using a capillary viscometer VPZh-1 (Ukraine) ($n = 5$) at 25.0 ± ± 1.0 °C maintained in a thermostat. The kinematic viscosity of the extracts was calculated by the formula:

$$V = \frac{g}{9{,}807} TK,$$

where K — the viscometer constant; V — the kinematic viscosity; T — the efflux time; g — the acceleration of gravity.

The specialized digital image analysis software Image-Pro Premier 9.0 (USA) was used for photo documentation and processing the data obtained in the histochemical analysis. Electrophoregram densitometry was conducted in the Sorbfil (RF) program. The data were statistically processed in Statistica 7.0 software (USA). Biochemical analysis of plant tissues was performed in four biological replicates ($n = 4$) with a significant difference to control at the significance level threshold $p \leq 0.05$.

Results and discussion

Nine medium variants (pH 5.9–6.0) based on MS, DKW and WPM with different modifications of growth regulators were used in selection of the optimal conditions for inducing callusogenesis in NRP and RP *Aesculus hippocastanum* forms (Table).

The leaf blades started to deform due to active cell proliferation and mesophyll dedifferentiation on the 14th day of cultivation after the introduction of

Nutrient medium composition for inducting callusogenesis in leaf blade tissues of resistant and non-resistant forms of Aesculus hippocastanum

Component	Concentration in variants, mg/L								
	1	2	3	4	5	6	7	8	9
Macro MS complex	100	–	–	–	–	100	–	–	–
Micro MS complex	1.0	–	–	–	–	1.0	–	–	–
Macro DKW complex	–	–	100	–	–	–	100	100	100
Micro DKW complex	–	–	1.0	–	–	–	1.0	1.0	1.0
Macro WPM complex	–	100	–	100	100	–	–	–	–
Micro WPM complex	–	1.0	–	1.0	1.0	–	–	–	–
MS vitamins	1.0	–	–	–	–	1.0	–	–	–
DKW vitamins	–	–	1.0	–	–	–	1.0	1.0	1.0
WPM vitamins	–	1.0	–	1.0	1.0	–	–	–	–
Fe chelate MS	5.0	–	–	–	–	–	–	–	5.0
Fe chelate DKW	–	–	5.0	–	–	5.0	5.0	5.0	–
Fe chelate WPM	–	5.0	–	5.0	5.0	–	–	–	–
Kinetine	–	–	–	–	–	–	–	–	0.5
NAA	–	–	–	–	–	2.0	2.0	2.0	–
BAP	–	–	–	–	–	1.0	1.0	0.5	–
2,4-D	–	–	–	–	–	–	–	–	3.0
IAA	–	–	–	–	1.0	–	–	–	–
TDZ	–	–	–	1.0	–	–	–	–	–
Myo-inositol, g/L	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Saccharose, g/L	30	30	30	30	30	30	30	30	30
Agar, g/L	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Callusogenesis	–	–	–	–	–	+	++	+	+++

Note: – callus did not form; + callus not morphogenic; ++ callus morphogenic on the 21th day; +++ callus morphogenic on the 28th day.

the explants *in vitro*. The active growth of the callus tissues began on the 20—21th day. The formation of morphogenic structures in calluses was observed on the 28th day of cultivation after the 2,4-D was added to medium to stimulate the formation of axial organs (№ 9, Table).

The halved (0.5 mg/L) BAP (6-benzylaminopurine) content in DKW medium reduced the augmentation of callus (№ 7, 8, Table). In this case, the callusogenesis was identified only in 10 % of explants of NRP and RP common horse chestnut forms. The most active callusogenesis was observed on DKW medium, with the addition of 0.5 mg/L kinetin and 3.0 mg/L 2,4-D. The reduction of concentration of 2,4-D to 2.5 mg/L accelerated the formation of callus tissues.

The obtained results and the data of other researchers confirm the ability

of this growth regulators combination to induce callusogenesis, organogenesis and embryogenesis in leaf explants *in vitro* [12]. The application of this combination allowed us to produce the *Aesculus hippocastanum* callus culture on media with different basic compositions of macro- and microelements (MS, DKW and WPM) supplemented with vitamins and other biologically-active components. Non-hormonal induction of callusogenesis was indicated only on the DKW medium. Tumefaction and deformation of the explants without callus formation was observed on WPM medium with 1.0 mg/L IAA (indole acetic acid) on the 6—8th days. The leaf blade fragments darkened with partial necrosis. No specific characters of the callus formation were observed when 1.0 mg/L TDZ was added to WFM medium.

Similar results were obtained when the explants of RP form were cultured. However, certain differences were observed. For example, on the DKW medium with the addition of 0.5 mg/L kinetin and 3.0 mg/L 2,4-D the first signs of NRP form explant callusogenesis appeared in 5-9 days later than for RP form. However, the formation of morphogenic callus followed by biomass growth was slower for the RP form.

The cells of the callus outer layers cleaved mostly periclinally at the beginning of morphogenesis. The resulting tissue of radiating structures, similar to the wound periderm was not dense and rather ordered (Fig. 1, a).

The intercellular space of the newly formed tissue contained a relatively high content of pectins. The proliferative active zones were deeper under the layers of the callus surface, forming strands of isodiametric cells with dense cytoplasm morphologically and topologically similar to the procambial cells.

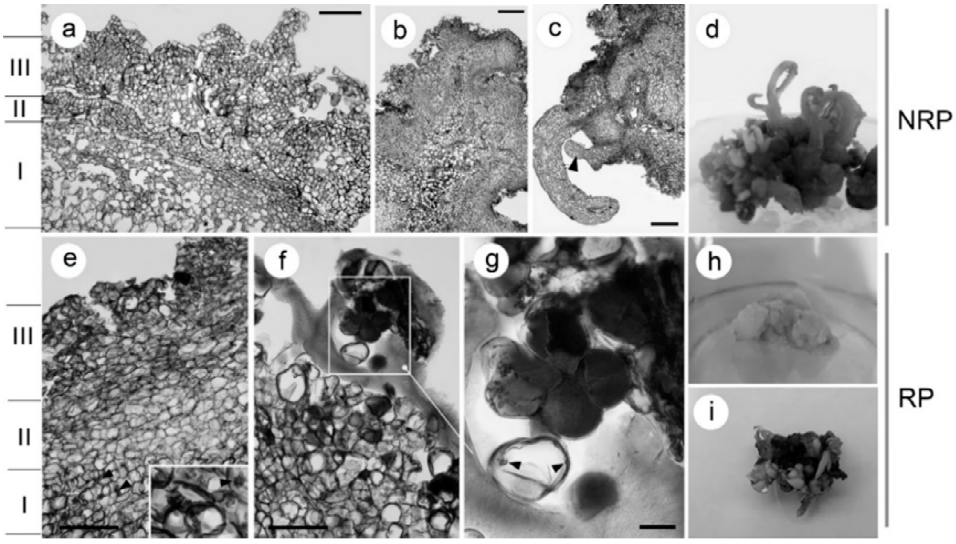

Fig. 1. Anatomic features and spatial-structural organization of callus tissue in the initial stages of morphogenesis of NRP and RP forms of *Aesculus hippocastanum*:

a — non-morphogenic callus tissues of NRP form, three areas: I (internal) — loose parenchymal cells with pronounced intercellular space; II (middle) — isodiametric cells with dense cytoplasm, actively dividing; III (external) — cells that form a periderm-similar structure; b, c — initial stages of the meristemoid formation; d — morphogenic callus; e — structure of the morphogenic callus (RP form), three areas: I (internal) — parenchymal cells with amorphous complexes of polysaccharides and phenols; II (middle) — parenchymal cells, active dividing; III (external) — cells that form a periderm-similar structure; f, g — thick-walled cells, filled with condensed tannins; h — non-morphogenic callus; i — morphogenic callus; scale: a — 100 μm; b, c — 200 μm; e, f — 50 μm; g — 15 μm

The area of loose parenchymal cells was formed in the calluses below. The shoot formation which was observed by the tissue differentiation typical for axial organs started in NPR form after the excrescence of parenchyma (Fig. 1). Thus, three structural and anatomical areas could be marked in NRP calluses: peridermal, procambial, parenchymal (Fig. 1, a).

The meristemoids gradually formed from undifferentiated callus cells under the influence of exogenous hormonal stimuli. They were followed by structures with anatomical structure typical for stalks (Fig. 1, b—d).

The general stages of shoot formation were overall similar in the calluses of RP common horse chestnut form (Fig. 1, h—i). However, significant micromorphological and histochemical cell differences preceded the active differentiation. Groups of idioblast cells with high polycondensated phenols (phlobaphenes) content were formed at the active growth stage of callus (Fig. 1, e—g). The idioblasts were mainly inside callus tissues. The size (4—15 μm) and quantity (up to 20) of polyphenolic aggregates in cells indicated an intensive polymerization of intracellular polyphenols, particularly catechins (flavan-3-ols and flavan-3,4-diols) [2]. Similar polyphenolic complexes are usually actively accumulated in the cells of stems and petioles of common horse chestnut *in vivo* [4, 14]

Structurally, three areas in calluses of RP form could be recognized (Fig. 1, e). The superficial part (area III) of callus was formed by cells filled with condensed tannins, with thick cell walls. Certain groups of idioblasts filled with a complex aggregate of polysaccharides and phenol compounds should be considered specifically. Those cells are structurally similar to microspores, partially divided into syncytia by internal membranes. Similar cell differentiation is described for *Quercus suber* L. microspores [9]. The authors describe this process as the beginning of proembryo formation. Indeed, the undifferentiated cells are surrounded by medium containing vitamins, hormones and other essential macro- and microelements. Such environment creates the conditions similar to the microsporangium chamber lined with tapetum.

A layer of parenchyma with thin cell walls underlined the outer layer of callus (area II). Numerous tracheate elements, capable of accelerated transportation of nutrients into tissues supporting cellular nutrition and differentiation, were formed among them. The internal area (area I) consisted of parenchymal cells. Many of them had in protoplasts the amorphous structures with polysaccharide and tannin complexes (Fig. 1, e, shown with arrows).

The above-mentioned features of cell differentiation and thickening of secondary cell walls are known to occur with the participation of oxidases, mainly peroxidases [1].

This property of enzymes has been confirmed in our experiments. The activity of peroxidases in the petioles, leaf blades and non-morphogenic calluses in the RP form was significantly higher (Fig. 2).

Evaluation of the peroxidases activity in leaf blades was hindered by tannins, which also are substrates for oxidation. Polyvinyl pyrrolidone (PVP), which partially binds phenolic compounds, was used to eliminate the competitive action of tannins to hydrochloric benzidine. For the reaction, 0.5 % PVP was determined to be optimal. Under such conditions, the activity of the anionic peroxidases in the leaf explant tissues of the RP form was 1.5 times higher than in that of NRP form. For tissues of nonmorphogenic calluses, this rate was five or more times higher than for the NRP form. Accordingly, it contributed to the oxidation of phenolic compounds and phytohormones, particulary the indolilacetic acid. Auxin oxidase activity of peroxidases is well

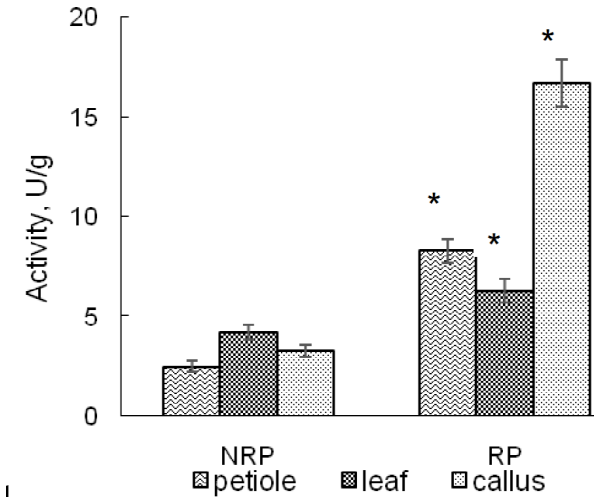

Fig. 2. Activity of the free and loosely-bound anionic peroxidases in petioles, leaf blades (0.5 % PVP) and non-morphogenic calluses of NRP and RP forms of *Aesculus hippocastanum*; * — the difference is valid to the NRP, $p \leq 0.05$

known. This factor could possibly be associated with the maximum potency of 2,4-D for the induction of callusogenesis in common horse chestnut. The synthetic growth regulator is stable, resistant to oxidation and causes stable cell proliferation. The majority of researchers used 2,4-D for the somatic embryogenesis [13, 15, 17].

Synthesis of endogenous plant hormones and phenolic compounds directly depends on the availability of free amino acids. According to the chromatographic profiling results, the callus tissues of RP form have a wider spectrum and higher cell concentration of free amino acids (including lysine, cysteine and glutamic acid) compared to NRP form (Fig. 3).

The latter two are a part of glutathione, an extremely important tripeptide protecting the cell and certain organoids against oxidative stress products.

The accumulation of lysine ($R_f \sim 0.30$) in the leaves and calluses is meta-

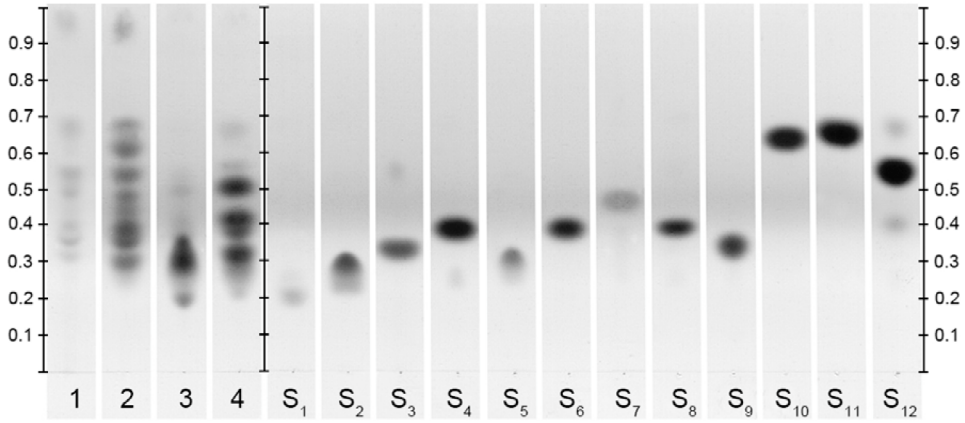

Fig. 3. Free amino acids profile in leaf blades (1, 2) and calluses (3, 4) of NRP (1, 3) and RP (2, 4) forms of *Aesculus hippocastanum*; standards: S_1 — cysteine; S_2 — lysine; S_3 — asparagine; S_4 — tryptophane; S_5 — arginine; S_6 — threonine; S_7 — proline; S_8 — glutamic acid; S_9 — serine; S_{10} — phenylalanine; S_{11} — isoleucine; S_{12} — valine

bolically linked to the formation of diaminopimelic acid and asparagine (R_f ~ 0.35). Increased asparagine content in the RP form, compared to NRP form, may indicate the activity of transaminase and asparagin synthase in the callus tissues responsible for the synthesis of this amino acid. The content of phenylalanine was insignificant in the non-morphogenic calluses of common horse chestnut. That amino acid is formed by the shikimate biosynthetic pathway in phosphoenolpyruvate and erythroze-4-phosphate condensation. Phenylalanine is a precursor of phenylpropanoid synthesis products. Its low amounts condition the possibility of insignificant content of phenolic compounds in calluses. At the same time the content of phenylalanine in calluses of the RP form was significantly higher on the chromatogram. The phenylalanine is needed for the synthesis of hydroxycinnamic acids, which are converted into lignin and deposited in cell walls. Presumably the relatively low pool of phenols in callus tissues is related to their peroxidase polymerization and lignification of cells in the outer area (III). Insufficient amount of tryptophan inhibits the synthesis of endogenous auxins, reduces proliferative activity and differentiation of cells. Conversely, the addition of amino acids may stimulate the formation of meristemoids and somatic embryos. Thus, the species-specific composition of free amino acids in common horse chestnut calluses necessitate adding the hydrolyzed casein (which contains a significant amount of tryptophan and tyrosine and is depleted in phenylalanine). In research Kiss and Radojevic [13, 17], the hydrolyzed casein was used to induce somatic embryogenesis in calluses of common horse chestnut.

Intense lignification of secondary cell walls and formation of idioblasts on the callus surface usually result from the high activity of anionic peroxidases. However, the explant tissues in the early stages of callusogenesis can probably serve as the source of phenolic compounds for the synthesis of lignin. The pool of free phenolic compounds reduced and lignification is inhibited after the formation of the primary callus. The thin, imperfectly-developed secondary parenchymal cell walls in the internal area of RP callus, which was formed later, can be explained by the decreased content of phenylalanine and phenolic compounds.

According to D'Costa [10], the viscosity of cell leaf juice may be the key factor limiting HCLM larval development. In our studies, this assumption was confirmed for the RP form of common horse chestnut. The kinematic viscosity of the RP form leaves (1.889 mm^2/s) was 1.53 times higher than that of NRP form (1.214 mm^2/s). In contrast, the content of phenolic substances was twice higher in the NRP form.

Thus, conditions for oxidative modification of intracellular polysaccharides can occur in leaves and calluses of the RP form, against the background of a relatively low pool of soluble phenolic substances, an increased content of free amino acids, and highly active anionic peroxidases. The substances similar to those filling the outer cells of calluses and the upper leaf epidermis of the RP form damaged by HLCM can also form as a result of oxidative stress. Such cellular processes can be considered as an induced immune reaction.

Conclusions. High activity of anionic peroxidases, which accelerate the lignification of the parenchyma cell walls in callus tissues, is one of the main experimentally determined properties of the callusogenesis in the common horse chestnut RP form. The lignification and polymerization of phenolic and other organic compounds are prerequisites for the formation of multiple idioblasts, which can mainly be found in the outer surface area of callus tissues and are not capable of division and formation of morphogenic structures.

The confirmed metabolic specificity of RP form can be explained by the relatively richer quantitative and qualitative composition of free amino acids in its tissues, compared to the NRP form. In general, the metabolism specificity of RP form callus tissues is a convenient model for studying the mechanisms of resistance against pathogens and pests of common horse chestnut.

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СПЕЦИФІЧНІСТЬ ДИФЕРЕНЦІАЦІЇ КЛІТИН У КАЛЮСАХ СТІЙКОЇ ДО КАШТАНОВОЇ МІНУЮЧОЇ МОЛІ ФОРМИ ГІРКОКАШТАНА ЗВИЧАЙНОГО IN VITRO

А.Ф. Ліханов[1], О.В. Оверченко[2], С.М. Костенко[2], О.В. Субін[2]

[1]Інститут еволюційної екології Національної академії наук України, Київ
[2]Національний університет біоресурсів і природокористування України, Київ

Досліджено специфіку калюсогенезу і диференціації клітин гіркокаштана звичайного нестійкої (NRP) і стійкої (RP) до каштанової мінуючої молі форм з урахуванням особливостей первинного і вторинного метаболізму рослинних тканин. З'ясовано, що ініціація калюсогенезу на листкових експлантатах спостерігається на поживному середовищі DKW з додаванням 0,5 мг/л кінетину і 3,0 мг/л 2,4-Д. Структурно в неморфогенних калюсах NRP і RP форм гіркокаштана звичайного виділено три зони. У RP форми поверхнева тканина (III зона) складалась із клітин з товстими клітинними стінками, заповненими конденсованими танінами. Під зовнішнім прошарком калюсу (II зона) виділено паренхіму з тонкими клітинними стінками. У цій зоні формувались численні трахеальні елементи, здатні пришвидшувати транспорт поживних речовин, сприяти живленню і диференціації клітин. Внутрішня (I зона) калюсу складалась із паренхімних клітин, у протопластах яких утворювались аморфні комплекси полісахаридів і танінів. Показано, що інтенсивне відкладання лігніну у вторинних стінках клітин поверхні калюсу RP форми відбувалось в умовах підвищеної активності аніонних пероксидаз, яка була в 5 і більше разів вищою, ніж у NRP. Відносно RP форм рослин гіркокаштана звичайного отримано підтвердження існуючого припущення про те, що життєздатність гусениць каштанової мінуючої молі залежить від в'язкості клітинного соку і мало пов'язана з вмістом у листках фенолів. Кінематична в'язкість водних екстрактів листків RP форми (1,889 мм^2/с) була в 1,53 раза вищою, ніж у NRP (1,214 мм^2/с). Вміст фенольних речовин, навпаки, в 2 рази вищий у листках NRP форми. Наявність у тканинах багатшого кількісного і якісного складу вільних амінокислот підтверджує положення про особливості первинного і вторинного метаболізму RP форми. Специфіка фізіологічних процесів калюсних тканин RP форми у цілому є зручною моделлю для дослідження механізмів стійкості рослин гіркокаштана звичайного до патогенів і шкідників.

СПЕЦИФИЧНОСТЬ ДИФФЕРЕНЦИАЦИИ КЛЕТОК В КАЛЛЮСАХ УСТОЙЧИВОЙ ПРОТИВ КАШТАНОВОЙ МИНИРУЮЩЕЙ МОЛИ ФОРМЫ КОНСКОГО КАШТАНА ОБЫКНОВЕННОГО IN VITRO

А.Ф. Лиханов[1], О.В. Оверченко[2], С.М. Костенко[2], А.В. Субин[2]

[1]Институт эволюционной экологии Национальной академии наук Украины, Киев
[2]Национальный университет биоресурсов и природопользования Украины, Киев

Исследована специфика каллюсогенеза и дифференциации клеток конского каштана обыкновенного неустойчивой (NRP) и устойчивой (RP) к каштановой минирующей моли форм с учетом особенностей первичного и вторичного метаболизма растительных тканей. Выяснено, что инициация каллюсогенеза на листовых эксплантатах наблюдается на питательной среде DKW с добавлением 0,5 мг/л кинетина и 3,0 мг/л 2,4-Д. Структурно в неморфогенных каллюсах NRP и RP форм конского каштана обыкновенного выделены три зоны. У RP формы поверхностная ткань (III зона) состояла из клеток с толстыми клеточными стенками, заполненными конденсированными таннинами. Под внешней прослойкой каллюса (II зона) выделена паренхима с тонкими клеточными стенками. В этой зоне формировались многочисленные трахеальные элементы, способные ускорить транспорт питательных веществ, содействовать питанию и дифференциации клеток. Внутренняя (I зона) каллюса состояла из паренхимных клеток, в протопластах которых образовывались аморфные комплексы полисахаридов и таннинов. Показано, что интенсивное отложение лигнина во вторичных стенках клеток поверхности каллюса RP формы происходило в условиях повышенной активности анионных пероксидаз, которая была в 5 и более раз выше, чем у NRP. Относительно RP форм растений конского каштана обыкновенного получено подтверждение существующего предположения о том, что жизнеспособность гусениц

каштановой минирующей моли зависит от вязкости клеточного сока и мало связана с содержанием в листьях фенолов. Кинематическая вязкость водных экстрактов листьев RP формы (1,889 мм^2/с) была в 1,53 раза выше, чем у NRP (1,214 мм^2/с). Содержание фенольных веществ, наоборот, в 2 раза выше в листьях NRP формы. Наличие в тканях более богатого количественного и качественного состава свободных аминокислот подтверждает положение об особенностях первичного и вторичного метаболизма RP формы. Специфика физиологических процессов каллюсных тканей RP формы в целом является удобной моделью для исследования механизмов устойчивости растений конского каштана обыкновенного к патогенам и вредителям.