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Comparison of Redox State of Cells of Tatar Buckwheat Morphogenic Calluses and Non-morphogenic Calluses Obtained from Them

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Abstract—Intracellular content of hydrogen peroxide and of the product of lipid peroxidation malonic dialdehyde as well as activity of antioxidant enzymes catalase, ascorbate peroxidase, and superoxide dismutase were studied in cells of morphogenic and derived from them non-morphogenic calluses of tatar buckwheat *Fagopyrum tataricum* L. Non-morphogenic calluses were characterized by significantly higher content of hydrogen peroxide and malonic dialdehyde, low catalase activity, and high activity of superoxide dismutase compared to morphogenic cultures. The results may indicate that cells of non-morphogenic calluses are in the state of continuous oxidative stress. Nevertheless, proliferative activity of non-morphogenic cultures and the biomass increase significantly exceeded these parameters in morphogenic calluses. An analogy is drawn between animal cancer cells and non-morphogenic plant calluses.

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Key words: morphogenic and non-morphogenic calluses, hydrogen peroxide, malonic dialdehyde, oxidative stress, antioxidant enzymes

One of main peculiarities of cultured cells is their high morphological and genetic variability. On one side, such variability can stimulate the emergence of mutants with properties valuable for selection, and on the other side, the accumulation with elongation of cultivation time of chromosomal and gene mutations can cause the loss of morphogenic ability in cultured cells. A possible reason for instability in plant cell cultures can be oxidative stress caused by cultivation conditions (explant damage, suboptimal hormone concentrations, their combinations, the presence of iron and prooxidants in the cultivation medium, osmotic shock, etc.) [1, 2]. Generation of such reactive oxygen species (ROS) as hydrogen peroxide, superoxide ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), and singlet oxygen ($^1O_2$) takes place during aerobic cell metabolism. Owing to high reactivity, ROS are able to react with and damage various macromolecules. Changes in nucleic acid composition not eliminated by reparation can be fixed during replication and finally realized in the form of gene or chromosomal mutations. Plant cells use different antioxidant systems for protection against damaging effects of ROS. These systems include non-enzymic antioxidants such as ascorbate, glutathione, tocopherol, and various phenolic compounds as well as antioxidant enzymes, among which the main are catalase, superoxide dismutase (SOD), and ascorbate peroxidase [3]. However, it is known that ROS can serve as signal molecules and they are necessary for launching a number of metabolic processes [4]. Therefore, it was concluded by Slezak et al. [5] that the plant antioxidant protective system is more inclined to cell redox-state control than to complete ROS elimination.

Nevertheless, works investigating cell pro- and antioxidant component effects on physiological–genetic stability of cultured cells are few. This is partially due to the absence of convenient models. One of a few works dealing with redox state in cultured cells exhibiting different morphogenic abilities is the work by Causevic et al. [6] that used organogenic, non-organogenic, and dedifferentiated calluses of sugar beet as an object of investigation. Results obtained by these authors are indicative of interrelationship between *in vitro* morphogenesis and cell redox state.

Morphogenic calluses of tatar buckwheat are unique cultures that retain morphological, morphogenic, and cytogenetic characteristics for a long time of cultivation

Abbreviations: LPO, lipid peroxidation; MDA, malonic dialdehyde; PECC, proembryonal cell complex; PMSF, phenylmethylsulfonyl fluoride; ROS, reactive oxygen species; SOD, superoxide dismutase.

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(up to 10 years) [7]. Non-morphogenic calluses emerge in this culture in the form of separate foci and extremely rarely (once per 30-40 passages). It is important to note that in the case of the friable callus formation on the morphogenic callus of tatar buckwheat it is possible to take the newly formed callus from the parental culture and to passage it separately. In this case, the morphogenic callus does not lose its regeneration potential, which makes possible comparative analysis of parental morphogenic line and derived non-morphogenic callus. We have selected four non-morphogenic lines from four different lines of the tatar buckwheat morphogenic callus.

It was shown earlier [8, 9] that intracellular content of hydrogen peroxide in non-morphogenic cultures of tatar buckwheat exceeded that in morphogenic cultures. It was supposed that ROS accumulation in the non-morphogenic culture cells could be caused by reduced activity of antioxidant enzymes. The goal of this work was to investigate the activities of a number of antioxidant enzymes (SOD, catalase, and ascorbate peroxidase) as well as of intracellular content of hydrogen peroxide and of the level of the malonic dialdehyde (MDA), and lipid peroxidation (LPO) product in morphogenic calluses of tatar buckwheat and non-morphogenic calluses selected from them.

MATERIALS AND METHODS

Calluses of tatar buckwheat *Fagopyrum tataricum* (L.) Gaertn. were used in this work. Morphogenic nodular calluses were obtained from immature embryos and consisted of proembryonal cell complexes (PECCs) and "soft" callus regions; we described earlier the morphology and production of such cultures [10, 11]. The bulk of PECC cells forms during loosening a "soft" callus whose long balloon-like cells have weak intercellular contacts are highly lignified and not capable of subsequent cell division [7]. Non-morphogenic calluses were selected as clones formed on actively dividing morphogenic calluses and differed from the "soft" callus by cytological–biochemical and morphological characteristics [7]. Morphogenic lines 1-5, 1-8, 1-10, 2-6 and non-morphogenic lines 1-5p, 1-8p, 1-10p, 2-6p obtained from them were used in this work. Callus cultures were maintained in a thermostat at 26 ± 2°C in the dark on callus-producing RX medium [10] containing Gamborg's B5 basal salt mixture [12] with addition of 2 mg/liter thiamine, 1 mg/liter pyridoxine, 1 mg/liter nicotinic acid, 2 g/liter casein hydrolysate, 2 mg/liter 2,4-dichlorophenoxyacetic acid, 0.5 mg/liter indolylacetic acid, 0.5 mg/liter naphthylacetic acid, and 0.2 mg/liter kinetin. Non-morphogenic calluses were replated once in two weeks, and morphogenic calluses were subcultured once in four weeks. To determine morphogenic potential, callus cultures were transferred on hormone-free MS medium [13] and grown in the light (5000 lx) under conditions of 16/8 h photoperiod and 25°C. The ability to form somatic embryos was registered.

Growth character of callus culture was estimated by the fresh tissue mass increase in selected times.

Cytogenetic preparations. Callus was incubated in Clark's fixative for 4-6 h. The fixative was washed off with 96% ethanol and the material was kept in 70% ethanol until investigation. Chromosomes were stained with 2% propionic orcein (Serva, Germany). Preparations were analyzed using a Jenamed microscope (Carl Zeiss, Germany), photographed using a Nikon CoolPix 4500 digital camera, and processed using the Adobe PhotoShop 7.0 program. In mitotic index determination, no less than 5000 cells were counted per fixation point. Chromosome numbers were counted in at least 150 metaphase plates with a good scatter of chromosomes.

Hydrogen peroxide content was determined spectrophotometrically according to Bellincampi et al. [14]. Callus tissue (200-300 mg) was ground with 1.0-1.5 ml cold acetone and the resulting homogenate was centrifuged (5 min, 12,000*g*). Then the supernatant was taken and used for analysis. Equal volumes of the extract were introduced into tubes together with reagent consisting of 0.5 mM $FeSO_4(NH_4)_2SO_4$, 50 mM xylenol orange (Sigma, USA), 200 mM sorbitol, and 50 mM H_2SO_4. Absorption was measured at 560 nm using a mixture of equal parts of the reagent and pure solvent as control. Hydrogen peroxide content was determined using a calibration curve plotted based on known hydrogen peroxide concentrations.

Determination of catalase (EC 1.11.1.16) activity. The spectrophotometric technique proposed by Aebi [15] was used. The method is based on determination of the rate of hydrogen peroxide decomposition by catalase of the sample used with formation of water and oxygen. A weighed sample of callus tissue was ground in a cooled mortar in 50 mM phosphate buffer, pH 7.8, containing 1 mM phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged for 5 min at 12,000*g* and then filtered through a Millipore ultrafilter with pore diameter 0.22 μm. Catalase activity was determined from the change in absorption at 240 nm due to hydrogen peroxide decomposition. The reaction mixture contained 2.978 ml buffer, pH 7.0, and 0.005 ml extract. The reaction was started by addition of 0.02 ml 0.6 M hydrogen peroxide.

Accumulation of the lipid peroxidation product malonic dialdehyde was monitored by reaction with thiobarbituric acid [16]. Callus tissue (500 mg) was ground with 1.0-1.5 ml 1% Triton X-100, the resulting homogenate was centrifuged for 5 min at 12,000*g*, and then the supernatant was taken and used for analysis. A 0.5 ml sample of supernatant in a tube was supplemented in succession with 0.5 ml 1% Triton X-100 solution, 0.2 ml 0.6 M HCl, and 0.8 ml 0.06 M working solution of thiobarbituric acid (864 mg thiobarbituric acid (4,6-dihydroxy-2-mercaptopyrimidine; Alfa Aesar, Germany) was dissolved in 100 ml 1% Triton X-100 with 50% ethanol). The tube

with this mixture was heated in a boiling water bath for 15 min. The tube was cooled at 15°C for 30 min. For stain stabilization after cooling, 0.1 ml 5 mM EDTA and 1 ml 96% ethanol were added. A control tube contained the same solutions except thiobarbituric acid. Absorbance was measured on a spectrophotometer at 532 nm as well at 600 nm for correction of nonspecific absorption [17]. For calculation of MDA content, absorption coefficient $\varepsilon = 155\ mM^{-1} \cdot cm^{-1}$ was used.

SOD (EC 1.15.1.1) activity was determined as described by Polesskaya et al. [18]. Total SOD activity was determined by the ability of the enzyme to inhibit photochemical reduction of nitroblue tetrazolium. Reaction mixture (3 ml) contained K-Na-phosphate buffer (50 mM, pH 7.8), methionine (13 mM), riboflavin (2 μM), *p*-nitroblue tetrazolium (Sigma, Germany) (63 μM), EDTA (0.1 mM), and 0.05 ml of the enzyme extract. The reaction proceeded for 15 min under illumination by luminescent lamps of total power 36 W. The complete reaction mixture incubated in the dark served as the dark control. The light control was complete enzyme-free reaction medium in which maximal staining was developed. The reaction was interrupted by switching off the light and transferring the sample in the dark. Absorption was determined at 560 nm. The amount of SOD able to inhibit the nitroblue tetrazolium reduction by 50% was taken as the enzyme activity unit. The SOD activity was expressed in relative units per gram dry weight.

Activity of ascorbate peroxidase (EC 1.11.1.7) was determined as described by Verma and Dubey [19]. A weighed sample of callus tissue was ground in 50 mM K-phosphate buffer, pH 7.8, 1 mM PMSF, 1 mM ascorbic acid, and 1% polyvinylpyrrolidone. The homogenate was centrifuged for 5 min at 12,000*g*, and the supernatant was used for analysis. The reaction mixture contained K-phosphate buffer, pH 7.0, 0.2 mM ascorbic acid, 0.2 mM EDTA, and enzymic extract. The reaction was initiated by addition of 20 μM hydrogen peroxide. Absorption was measured at 290 nm during 120 sec with 1 sec intervals on a Lambda 25 spectrophotometer (Perkin Elmer, USA).

Data of three independent experiments with three biological replications in each are shown. Figures and tables show arithmetic means of values and arithmetic mean errors are given to show the scatter of experimental data.

RESULTS

Morphogenic nodular calluses were obtained from immature embryos and consisted of PECCs and the soft callus regions (Fig. 1a); the morphology and obtaining of

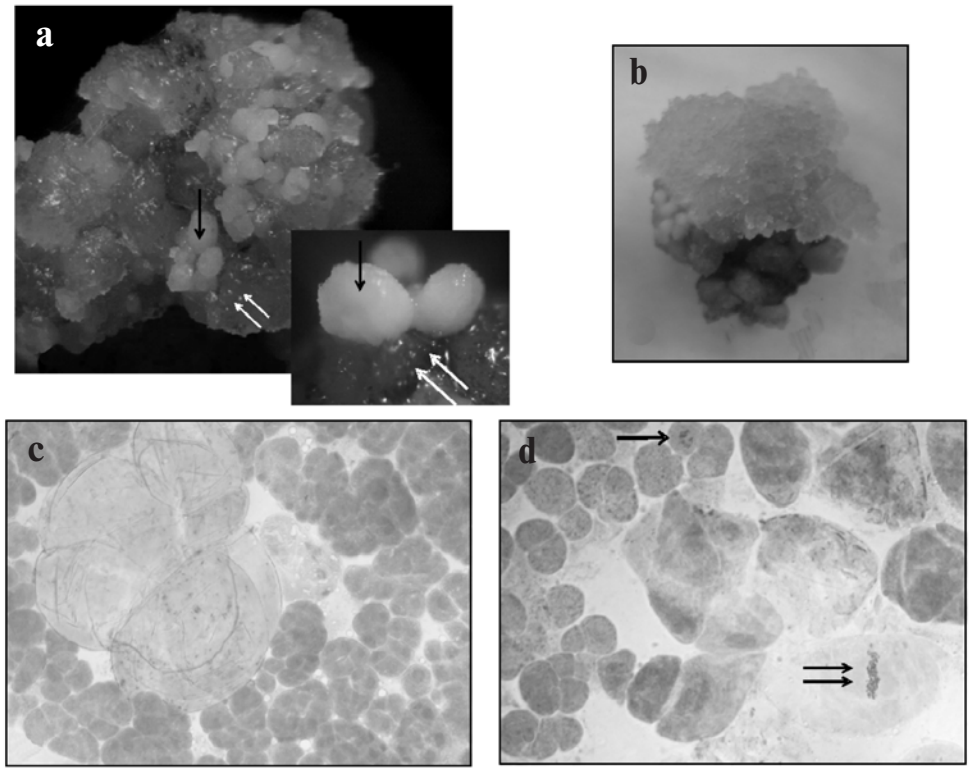

Fig. 1. Formation of non-morphogenic callus friable clones on the tatar buckwheat morphogenic callus. a) Appearance of the tatar buckwheat morphogenic callus: a single arrow points to proembriogenic cell complexes, two arrows point to "soft" callus; b) formation of a friable clone; c, d) magnification ×300, a single arrow points to the morphogenic callus cells, two arrows point to the non-morphogenic callus cells.

such cultures were described previously [10, 11, 20]. Formation of friable non-morphogenic calluses proceeded in the form of clones on already formed and passaged morphogenic calluses (Fig. 1b). Morphologically different clones could be easily separated from "parental" tissue. Only just formed clones several millimeters in diameter differed from the morphogenic parental culture by cell dimensions and by significantly higher chromosome numbers (Fig. 1, c and d). The cytological preparation (Fig. 1d) presents well-distinguished small diploid cells of morphogenic callus with dense cytoplasm and large highly vacuolated polyploid cells of non-morphogenic callus. Cytogenetic analysis of morphogenic cultures and derived from them non-morphogenic calluses revealed differences in chromosome numbers already at early stages of their formation. The modal ploidy class in morphogenic calluses was represented by diploid cells whose part made up 95-98% (Fig. 2). Such event was observed for all studied lines of morphogenic callus. Non-morphogenic cultures consisted mainly of polyploid and aneuploid cells, and the fraction of cells with a diploid number of chromosomes did not exceed 6%. It is interesting to note in this case that lines with prevalent number of polyploid cells (lines 1-8p and 2-6p) and lines (1-5p and 1-10p) in which the fraction of polyploid and aneuploid cells was practically identical were revealed (Fig. 2).

Parental and daughter lines differed by the character of biomass increase (Fig. 3). Rapid biomass increase was registered for non-morphogenic calluses, which reached fivefold increase over the initial level by 14 day of cultivation. Morphogenic calluses grew at a significantly lower rate and in 2 weeks they only doubled their biomass.

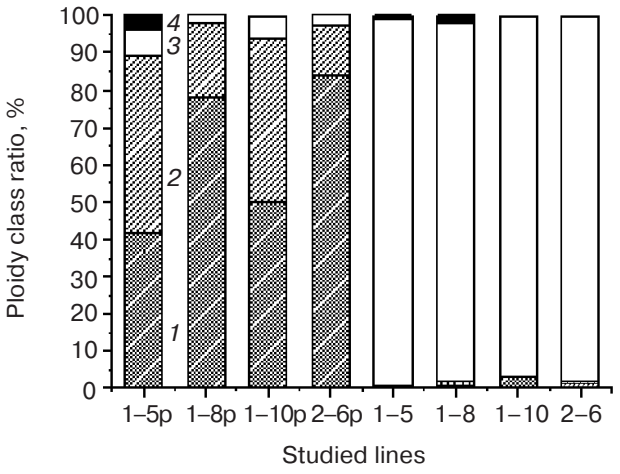

Fig. 2. Ploidy class ratio in tatar buckwheat morphogenic and non-morphogenic calluses, %; *1*) polyploid cells; *2*) aneuploid cells; *3*) diploid cells; *4*) haploid cells. Abscissa axis shows studied lines; ordinate axis shows ploidy class ratio, %.

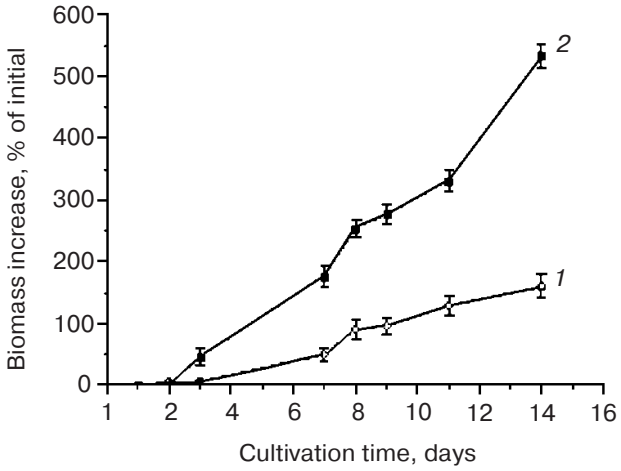

Fig. 3. Biomass increase during passage of tatar buckwheat morphogenic and non-morphogenic calluses: *1*) morphogenic callus; *2*) non-morphogenic callus. Abscissa axis shows duration of culture, days; ordinate axis shows biomass increase, % of initial.

Differences in proliferative activity of morphogenic and non-morphogenic calluses were also observed. Non-morphogenic calluses had a single peak of mitotic activity at the beginning of the cultivation cycle, and the fraction of dividing cells was 3-4.26% depending on the line (Fig. 4, a, d, g). Several peaks of proliferative activity were observed for morphogenic calluses, which may be due to the processes of PECC re-initiation, and maximal level of mitotic activity was 2.37% (Fig. 4, a, d, g).

Investigation of intracellular hydrogen peroxide content revealed differences both between morphogenic and non-morphogenic calluses as well as between lines of similar morphogenic activity. It was found that increased content of hydrogen peroxide is characteristic of all studied lines of non-morphogenic calluses compared to parental morphogenic lines. Differences in hydrogen peroxide content between morphogenic and derived from them non-morphogenic lines were from 1.5 to 15 times (Fig. 4, b, e, h). It is interesting that in all non-morphogenic cultures a sharp increase in hydrogen peroxide content was observed by the third day of cultivation, which was followed by a fall on the fourth day and then by increase again. In morphogenic calluses, increase in intracellular hydrogen peroxide content was observed at the second-to-fourth days (lines 1-8, 2-6) and at the fourth-to-seventh days of cultivation (line 1-10). In this case, the peak was more smoothed and extended compared to that in non-morphogenic cultures and values of hydrogen peroxide content were 1.5-2.4-fold lower.

Studying the LPO level in callus cells showed that the highest MDA content is characteristic of non-morphogenic calluses (Fig. 4, c, f, i). In this case, maximal levels in non-morphogenic calluses were 10-13 nmol/g dry weight, whereas the MDA content in morphogenic calluses did not exceed 6 nmol/g dry weight. It should be

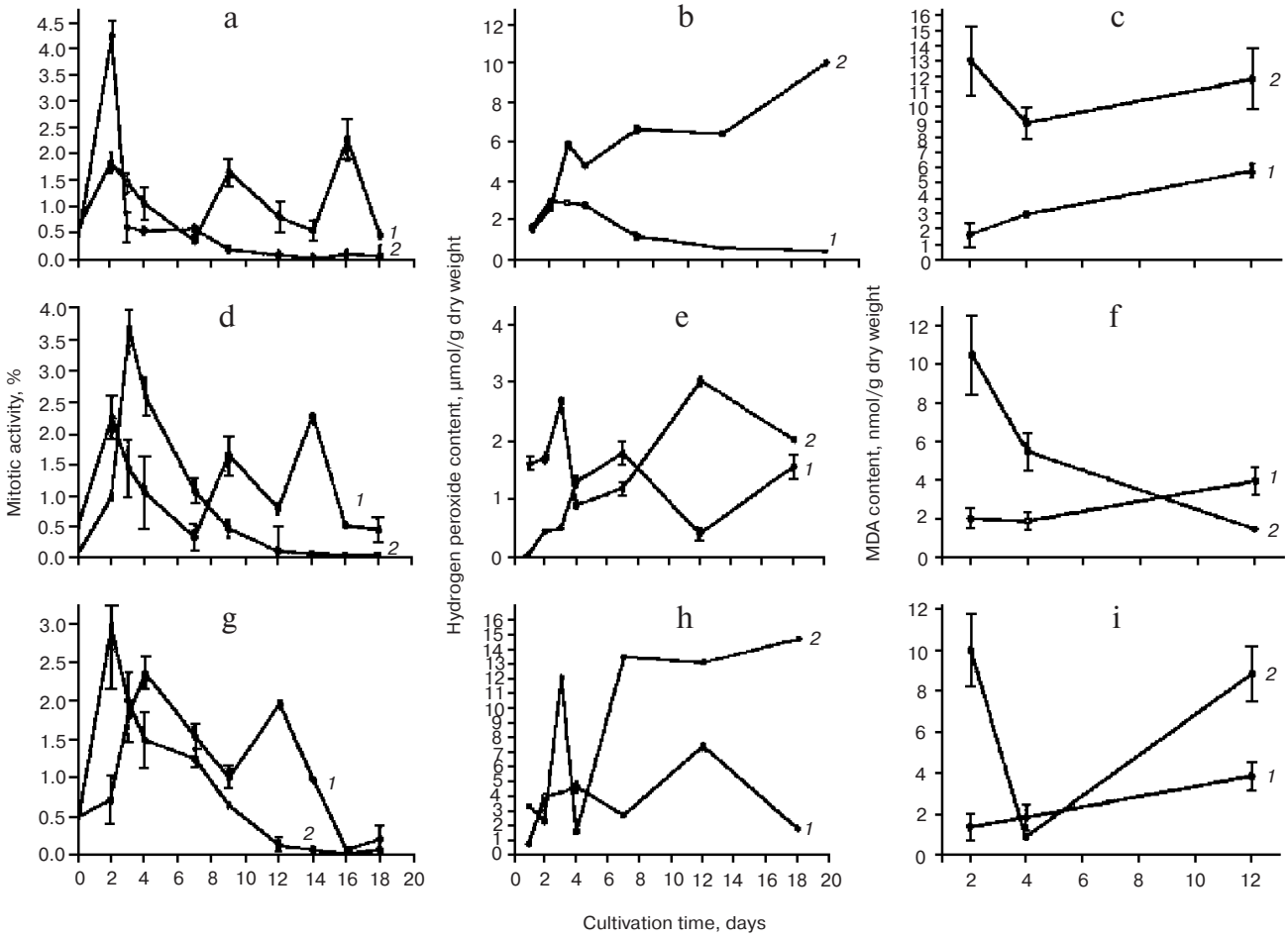

Fig. 4. Dynamics of proliferative activity (a, d, g; ordinate axis shows mitotic activity, %), hydrogen peroxide content (b, e, h; ordinate axis shows hydrogen peroxide content, μmol/g dry weight) and MDA content (c, f, i; ordinate axis shows MDA content, nmol/g dry weight) in cells of tatar buckwheat morphogenic and non-morphogenic callus cultures: *1*) morphogenic lines; *2*) non-morphogenic lines. a, b, c) Lines 1-8 and 1-8p; d, e, f) lines 1-10 and 1-10p; g, h, i) lines 2-6 and 2-6p. Abscissa axis shows cultivation time, days.

Activity of antioxidant enzymes and hydrogen peroxide and MDA content in different cell populations of tatar buckwheat morphogenic callus line 1-8

Studied parameter \ Cell population	PECCs	"Soft" callus
SOD, relative units/ g dry weight	41.48 ± 1.58	62.64 ± 1.62
Catalase, relative units/ g dry weight	162 ± 25	337 ± 33
Ascorbate peroxidase, relative units/g dry weight	20.3 ± 1.2	38.5 ± 1.6
Hydrogen peroxide, μmol/g dry weight	4.9 ± 0.73	5 ± 0.61
MDA, nmol/g dry weight	4 ± 0.33	11.3 ± 1

noted that the increase in MDA content in the non-morphogenic callus cells was not due to culture aging and was observed already on the second day of the passage. The MDA content in morphogenic calluses increased closer to the second half of the passage.

It was found that activity of antioxidant enzymes in callus cells of different morphogenetic ability significantly varies (Fig. 5, a-i). Nevertheless, the following regularity was observed within pairs of parental morphogenic callus with selected non-morphogenic callus. Catalase activity in the morphogenic callus cells exceeded that in cells of non-morphogenic calluses (Fig. 5, a, d, g), whereas ascorbate peroxidase, on the contrary, exhibited high activity in cells of non-morphogenic calluses (Fig. 5, b, e, h). On the average, SOD activity in cells of non-morphogenic calluses exceeded by 2.5 times that in parental lines (Fig. 5, c, f, i). An exception was the line of morphogenic callus 2-6 characterized by increased SOD activity and lowered activities of catalase and ascorbate

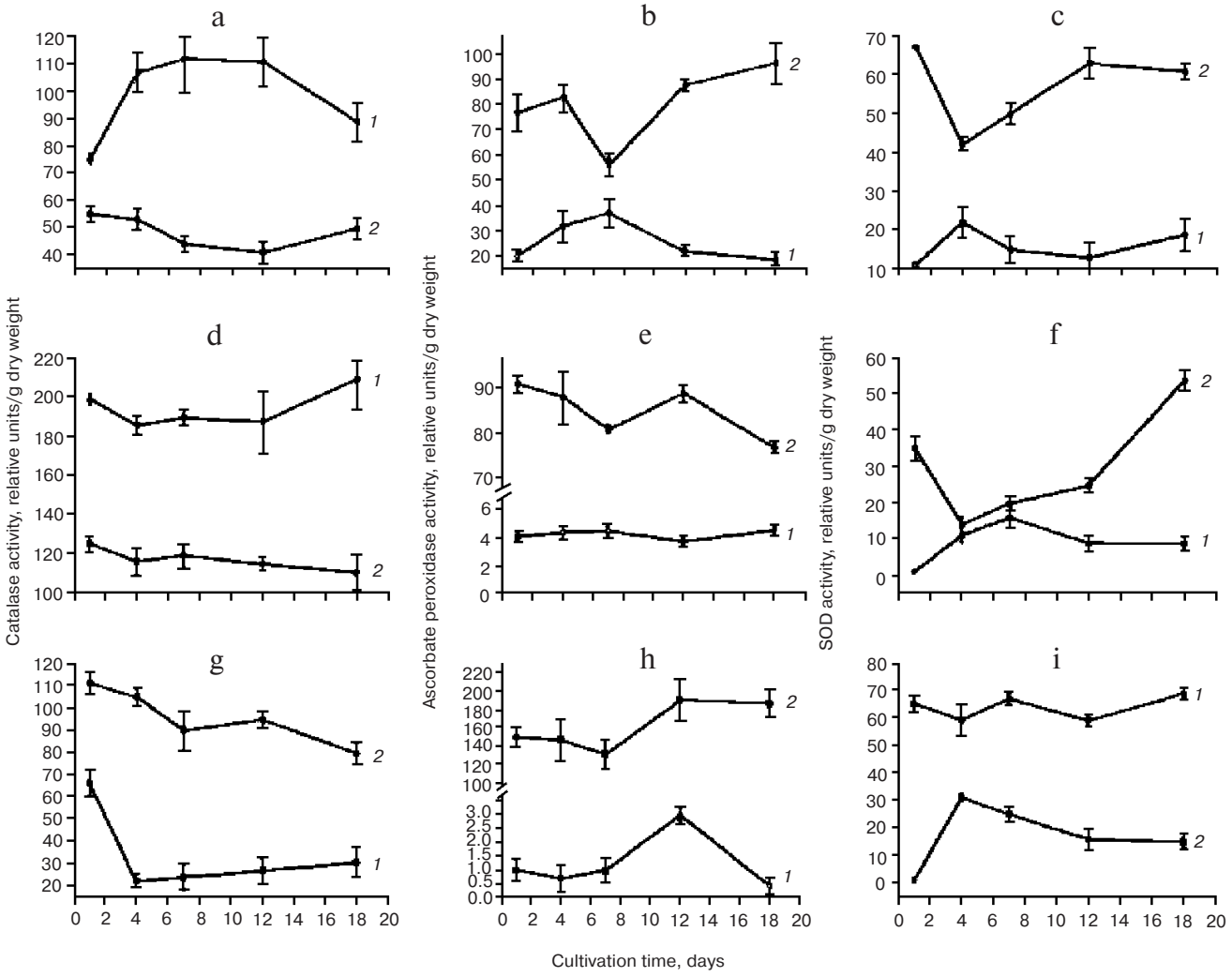

Fig. 5. Dynamics of antioxidant enzyme activities: catalase (a, d, g), ascorbate peroxidase (b, e, h), and SOD (c, f, i) in cells of the tatar buckwheat morphogenic and non-morphogenic calluses: *1*) morphogenic calluses; *2*) non-morphogenic calluses. a, b, c) Lines 1-8 and 1-8p; d, e, f) lines 1-10 and 1-10p; g, h, i) lines 2-6 and 2-6p. Abscissa axis shows cultivation time, days; ordinate axis shows enzyme activity, relative units/g dry weight.

peroxidase, whereas non-morphogenic callus of this line, on the contrary, exhibited high activity of catalase and ascorbate peroxidase and low SOD activity (Fig. 5, g, h, i). Nevertheless, maximal level of intracellular hydrogen peroxide in non-morphogenic line 2-6p exceeded that in parental morphogenic line 2-6 (Fig. 4h).

Since morphogenic callus of tatar buckwheat is a heterogeneous culture consisting of PECCs and of the "soft" callus formed during loosening of the latter, it seemed interesting to study redox state in different cell populations. It was found that LPO content was significantly higher in the "soft" callus cells than in PECC cells (table), whereas hydrogen peroxide content in PECCs and "soft" callus cells was identical. Besides, we observed an increase in catalase, ascorbate peroxidase, and SOD activities in the "soft" callus cells (table).

DISCUSSION

Morphogenic cultures of tatar buckwheat can be considered as morphogenetically stable cultures that retain the capability of plant regeneration during prolonged cultivation. Such cultures mainly contain diploid cells. Formation of the non-morphogenic callus friable clones on morphogenic plants of tatar buckwheat is an extremely rare event that can be considered as a manifestation of *in vitro* instability expressed in drastic alteration of physiological–genetic characteristics of the culture (changes in cell dimensions, chromosome number, proliferative activity, and regeneration ability). Note that the appearance in morphogenic calluses of tatar buckwheat of haploid and polyploid cells at a low frequency may be due to spontaneous mutations. Their small fraction is

indicative of limited division or low viability of such cells. Probably in very rare cases, polyploid cells are proliferation-competent, which results in emergence of foci of non-morphogenic callus. Oxidative stress in the "soft" callus cells, detected by enhancement of LPO processes and significant SOD activation, might be responsible for emergence of friable clones because it is known that mutations can be induced by oxidative stress [21]. We found that both polyploid and aneuploid cells are present in the tatar buckwheat non-morphogenic calluses. A hypothesis that explains cell transition from polyploidy to aneuploidy was described in a review [22]. We have shown that polyploid cells are prevalent in two lines, whereas the amount of polyploid and aneuploid cells in another two lines is practically equal. Possibly, we observe a decrease in the number of polyploid cells due to their transition to aneuploid state.

As is known, genomic alterations lead to changes in gene expression [23, 24] and, as a result, to metabolic rearrangements. A change in gene expression can be caused both by gene dose increase [25] and by change in a number of regulators, such as transcription factors [26, 27], as well as of signal molecules. Many recent works show that hydrogen peroxide serves as regulator in cell signaling [5, 28]. In this case, hydrogen peroxide activates transcription factors [29] or influences their expression [30], activates MAP kinases [31], and inhibits tyrosine phosphatases [32]. It can be supposed that the observed increase in the intracellular content of hydrogen peroxide might be a necessary condition for regulation of polyploid genome of non-morphogenic calluses. At the same time, besides increased intracellular content of hydrogen peroxide in the non-morphogenic callus cells, we observed enhancement of the LPO process, which is, like hydrogen peroxide, a marker of oxidative stress [33]. LPO is initiated by ROS, and its development has chain-like character. It can result in formation of aldehydes such as 4-hydroxy-2-nonenal and MDA. Products of aldehyde decomposition are toxic because they form conjugates with DNA and proteins [34]. Therefore, the observed increase in intracellular content of hydrogen peroxide and MDA might indicate that the non-morphogenic callus cells exist under conditions of continuous oxidative stress. Besides, high SOD activity can be indicative of higher $O_2^{\cdot-}$ generation in non-morphogenic callus cells, whereas reduced catalase activity in non-morphogenic calluses can be explained by the necessity of maintaining high intracellular hydrogen peroxide content. It should be noted that conditions of *in vitro* cultivation cause stress in both plant and animal cells [35, 36]. Therefore, it is necessary for cells to form mechanisms of adaptation to ROS excess in order to survive and grow *in vitro*. Protection against ROS can be achieved by activation of antioxidant enzymes. There are data in the literature concerning differences in the antioxidant enzyme activities in totipotent and non-totipotent cells. It was found that in the case of tobacco protoplasts the decrease in totipotency correlated with lowering ascorbate peroxidase, SOD, and glutathione reductase activities [37, 38]; in non-regenerating wheat calluses catalase activity was lower than that in regenerating calluses [39]; in rice suspension culture that lost ability for morphogenesis, catalase and peroxidase activities were significantly lower [40]. On the contrary, an increase in catalase activity was recorded in grape non-totipotent protoplasts [41]. We found in our investigations that catalase activity in non-morphogenic calluses was lower, whereas SOD and ascorbate peroxidase activities were higher than in morphogenic calluses. High SOD activity in non-morphogenic calluses can be one of the factors responsible for higher intracellular content of hydrogen peroxide. Since SOD contributes to hydrogen peroxide formation, efficient protection against ROS will be carried out only in the case of accompanying increase of catalase and ascorbate peroxidase activities [42, 43]. It is important that we observed the inverse situation for the pair of lines 2-6 and 2-6p: in the morphogenic line, catalase activity was significantly lower and SOD activity was higher than in non-morphogenic line. It can be supposed that in this case the low hydrogen peroxide content in the morphogenic line cells is provided by components of non-enzymic antioxidant protection. Thus, we have shown that in morphogenic and non-morphogenic tatar buckwheat cultures enzymes involved in antioxidant protection are expressed differently. Evidently, metabolism of non-morphogenic cultures differs from that of morphogenic cultures. Gaspar et al. [2] showed that increased hydrogen peroxide content in animal and plant tumor cells can be the result of anomalous metabolism (alterations of metabolism of sugars, polyamines, nitrogen, heme-containing proteins) and simultaneously a necessary factor of its maintenance. It is known that ROS effects on cells are strictly concentration-dependent: low ROS concentrations stimulate proliferation, medium concentration cause aging, and high cause cell death [44, 45]. Nevertheless, we did not observe a clear correlation between intracellular hydrogen peroxide content and proliferative activity. In non-morphogenic calluses the peak of proliferative activity preceded the increase in the hydrogen peroxide content in the cells (lines 1-8p, 1-5p, and 2-6p) or coincided with it (line 1-10p). In this case following increase in intracellular hydrogen peroxide content in non-morphogenic cultures was not associated with cell division. In morphogenic lines 2-6 and 1-8 the first peak of mitotic activity also coincided with intracellular hydrogen peroxide content, whereas in the line 1-10 the peak of proliferative activity was registered on the second day and the first peak of hydrogen peroxide content was observed only on the seventh day.

According to Halliwell [46], cells adapted to oxidative stress can further use ROS. For example, some malignant animal cells are able to use *in vitro* ROS for prolifer-

ation promotion and apoptosis suppression [47, 48]. In the case of chronic inflammation, excessive ROS formation or defects in ROS detoxification result in cancer promotion via activation of transcription factors or inhibition of tumor suppressor genes [49]. On the other side, ROS in appreciable concentrations inhibit cancer cell growth via stimulation of proapoptotic signals [50], thus making cancer cells hypersensitive to stressors. This can be explained by the fact that in animal cancer cells, unlike normal ones, intracellular H_2O_2 concentrations are closer to toxic and therefore further increase in H_2O_2 concentration in response to certain effects switches cell development to apoptosis [51]. While exposures at low ROS concentrations stimulate proliferation of normal cells, the same treatments cause growth cessation and death of cancer cells [49]. The existing hypothesis that cells of non-morphogenic callus cultures by their cytological–biochemical characteristics are close to animal cancer cells has recently received significant experimental confirmation [2, 52]. Gaspar et al. [2] noticed that habituated non-organogenic cultures are in conditions of continuous oxidative stress (because increased content of hydrogen peroxide, low catalase activity, and high SOD activity are registered in these cultures) like animal cancer cells, and both actively proliferate. Our results show that unlike morphogenic calluses, cells of the tatar buckwheat non-morphogenic calluses are characterized by high intracellular concentrations of hydrogen peroxide and MDA, but they exhibit more active proliferation and biomass accumulation. We showed previously that non-morphogenic calluses are much more sensitive to treatment by salicylic acid inducing oxidative stress [53]. Hypersensitivity to stressors can be also in favor of a community of regulatory processes (or their disturbance/alteration) in plant and animal cancer cells.

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