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TARGETED PROTECTION OF MITOCHONDRIA OF MESOPHYLL CELLS IN TRANSGENIC *CYP11A1* cDNA EXPRESSING TOBACCO PLANT LEAVES AFTER NaCl-INDUCED STRESS DAMAGE

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Recently we have showed that the expression of the mammalian CYP11A1 *cDNA in plants confers their resistance to abiotic and biotic stresses. To determine the role of heterologous expression of cytochrome P450scc cDNA in resistance to ROS (radical oxygen species) dependent abiotic stresses, the structural changes of mitochondria and peroxisomes were studied under 150 mM NaCl-induced 14-day salinity treatment on juvenile tobacco plants in* in vitro *culture. Ultrastructural analysis of mesophyll cells of transgenic tobacco leaves constitutively expressing* CYP11A1 *cDNA was performed. Under NaCl stress, a change in shape from rounded to elongated, reduced section area, formation of branched mitochondria, as well as the emergence of triangular and rhomboid cristae, densification of a mitochondrial matrix, increase in density of contrasting membranes and their thickness were observed in non-transgenic plants. Transgenic plants without stress applied had mitochondria with rounded and elongated shape, twice as small as in non-transgenic plants, with a dense matrix and sinuous cristae. Surprisingly, the effect of NaCl led to increase in size of mitochondria by 1.5 times, decomposition of matrix and the emergence in organelles of light zones presumably containing mitochondrial DNA strands. Thus, the structural organisation of transgenic plant mitochondria under salinity treatment was comparable to that of non-transgenic plants under native conditions. It was also noted that the transgenic plant peroxisomes differed in non-transgenic tobacco both in normal condition and under the action of NaCl. The observed differences in ultrastructural organisation of mitochondria not only support our earlier notion about successful incorporation of the mature P450scc into this organelle, but for the first time demonstrate that the mammalian CYP11A1 signal peptide sequence could be efficiently used in the formation of targeted mitochondria protection of plants from salinity-induced damage.*

Key words: CYP11A1*, cytochrome P450scc, salinity, mitochondrion ultrastructure, transgenic plants.*

INTRODUCTION

NaCl-tolerant plants have a series of adaptations to acclimate to salinity, including morphological, physiological, and biochemical changes (Acosta-Motos *et al*., 2017). Electron microscopic studies showed that the NaCl action results in deficiency of mitochondrial cristae in *Oryza sativa* L. The mitochondria also become swelled and their matrix appears pale in salt-treated plants, compared to control conditions (Rahman *et al*., 2000). Our previous experiments,

NaCl-salinity treatment of tomato resulted in a rounded shape of mitochondria, which contained a light density matrix and triangular cristae in mesophyll cells of wild type regenerants (Baranova *et al*., 2014). In addition to chloroplasts, mitochondria and peroxisomes are also considered to be major sites of the ROS generation (Corpas *et al*., 1999; Foyer and Noctor, 2000; Møller, 2001; del Rio *et al*., 2002). However, a role of mitochondrial and peroxisomal antioxidant systems in root protection against oxidative stress has hardly been investigated.

It is known that mitochondria are one of the oldest endosymbiotic organelles typical for all eukaryotic species. A number of sequences necessary for division and metabolism of mitochondria is encoded in the nuclear compartment of the genome (Hammani and Giegé, 2014). Mitochondria-directed sequences are provided with a signal that enables the passage of proteins into the mitochondria and controls the transfer of polypeptide fragments from the cytoplasm to the mitochondria (Lee *et al*., 2014; van Wijk *et al*., 2015). Due to the need for precise regulation of cellular metabolism processes at the single organelle level in the development of transgenic plants with increased resistance to abiotic and biotic stress by genetic engineering methods, use of characterised signal peptide sequences for targeting transit of proteins to the desirable cellular compartments seems to be the most promising solution (Gerszberg *et al*., 2017).

Recently, there have been some indications that salt stress induces an increase in antioxidant enzyme activity, including of SOD, APX, and MDHAR in chloroplasts/plastids, mitochondria, and peroxisomes in root and leaf cells (Mittova *et al*., 2004; Zlobin *et al*., 2017). This is associated with reduced oxidative stress indicators such as H_2O_2 content and lipid peroxidation in those organelles. At the same time, the increased salt-induced activity of a number of neutralising enzymes was not observed, which caused compartmental damage typical for oxidative stress. This was due to the increased H_2O_2 content and lipid peroxidation in organelles (Mittova *et al*., 2004). According to the authors, this differential response to the salinity stress of organelle antioxidant systems of two tomato varieties may indicate that corresponding antioxidant genes are unlikely to be regulated by salinity.

For most plants, the relationship between resistance to abiotic stress and changes in hormone levels was established (Verma *et al*., 2016). It is known that plant cells contain steroid hormones that perform various functions, but this has been insufficiently studied. Estranes, pregnanes and corticoids appear to have hormonal effects on plants. It has been established that steroids like 17β-estradiol, androsterone, testosterone and progesterone affect cell division, growth of roots and shoots, development of generative organs, flowering, growth of pollen tubes and callus proliferation producing specific effects on growth processes and ontogenesis of plants (Ylstra *et al*., 1995; Lindemann, 2015; Shpakovski *et al*., 2017). The main stages of biosynthesis and metabolism of steroid hormones in animals and brassinosteroids in plants involve catalysis of P450 cytochromes. In both living systems, enzymes like steroid-5α-reductase (5αR) and 3b-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase (3β-HSD) have been detected. It was also found that in plants, along with the most well-studied brassinosteroids, steroid hormones characteristic of animal cells are also present: progesterone, 17-hydroxyprogesterone, 16-dehydroxyprogesterone, and androstenedione (Simerskż *et al*., 2009; Pauli *et al*., 2010). Recently, it has been shown that transgenic plants transformed by cDNA of the CYP11A1 gene have increased resistance to a number of biotic and abiotic stress factors (Shematorova *et al*., 2014; Shpakovski *et al*., 2017).

Nonspecific resistance phenomena, triggering the ROS neutralising mechanism or regulating the osmotic pressure or binding and localisation of damaging ions in plants, are being intensively studied now (Wang *et al*., 2003; Rejeb *et al*., 2014; Saxena *et al*., 2017). Earlier we found that redirection of the cytoplasmic enzyme of Fe-dependent superoxide dismutase 1 into the plastid results in increased resistance to chloride and sulphate salinity (Baranova *et al*., 2011) and to *Phytophthora infestans* (Baranova *et al*., 2017), which clearly indicates the formation of nonspecific protection. At the same time, transgenic tobacco and tomato plants showed changes in the ultrastructural organisation of plastids and mitochondria manifested in relocation of thylakoids and cristae, respectively, and in the emergence of specific inclusions associated with responses to oxidative stress in the absence of its natural modulators (cold, UV, salinity, etc.) — increased plastoglobules in the plastid stroma, the presence of inclusions and the emergence of clearly pronounced triangular cristae in the mitochondria (Serenko *et al*., 2011).

In this work, we aim to determine whether the P450scc cytochrome targeting provided by the expression of the *CYP11A1* cDNA containing its own signal sequence can effectively protect the mitochondrial compartment of mesophyll cells of transgenic tobacco from the salinity-specific ultrastructure damages induced by cultivation in the presence of 150 mM NaCl.

MATERIALS AND METHODS

Plant material. In this work, we used the earlier seeds produced by us of tobacco plants *Nicotiana tabacum* L. of the T4 generation transformed by cDNA of the *CYP11A1* gene encoding the cytochrome P450scc from bovine adrenal cortex. The cDNA contained its own signal sequence responsible for targeting of protein product into the mitochondrion (Spivak *et al*., 2009; Spivak *et al*., 2010; Shematorova *et al*., 2014). Expression of cDNA of the *CYP11A1* gene was controlled by the cauliflower mosaic virus 35S promoter (Fig. 1). Different generations of the aforementioned transgenic plants used in the study were thoroughly described in our original patent applications (Kartel *et al*., 2004; Kartel *et al*., 2007) and also in numerous more later publications (Spivak *et al*., 2009; Spivak *et al*., 2010; Shematorova *et al*, 2014; Shpakovski *et al*, 2017), with the full sequence of the *CYP11A1* cDNA insert given in Shematorova *et al*., 2014.

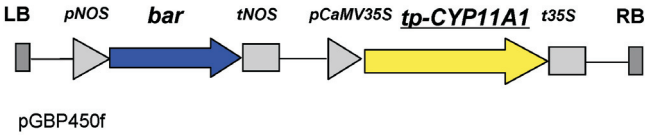

Fig. 1. Structure of the T-DNA region of the plasmid pGBP450f (pGreen-P450scc) used for tobacco transformation. LB, left border; *pNOS*, nopaline synthase promoter; bar, phosphinotricin acetylase gene from *Streptomyces hygroscopicus*; *tNOS*, nopaline synthase terminator sequence; *pCaMV*35S, constitutive promoter of 35S RNA of Cauliflower Mosaic Virus; *tp*, mammalian mitochondrial transition peptide coding sequence; *CYP11A1*, cDNA encoding cytochrome P450scc from the bovine adrenal cortex; *t35S*, 35S RNA terminator derived from Cauliflower Mosaic Virus; RB, right border.

Two homozygous transgenic tobacco lines, TR-2 and TR-7, used in the study were characterised previously in Shpakovski *et al*., 2017. The tobacco seeds were surface sterilised in 96% ethanol for 10 s and in 20% solution (v/v) of the commercial bleach 'Ace' with a few drops of Tween-20 for 15 min, then rinsed with sterilised distilled water six times for 1 min each. After surface sterilisation, the seeds were cultured on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) without growth regulators supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. The pH was adjusted to 5.8 before autoclaving. The cultures were maintained under 23 ± 1 °C, with fluorescence light (65 μmol m^2 s^{-1}) during the long-day photoperiod (16 h light/8 h dark).

Salinity treatment. The seeds of non-transgenic tobacco plants (*Nicotiana tabacum* cv. Petit Havana SR1) and two transgenic homozygous tobacco lines (TR-2 and TR-7, described in Shpakovski *et al*., 2017) of the T4 generation expressing *CYP11A1* cDNA, encoding cytochrome P450scc with mitochondrion signal sequence from the bovine adrenal cortex, were aseptically germinated on MS basal medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and 0.7% (w/v) agar. Fourteen-day exposure was used to study the NaCl effect in *in vitro* culture on transgenic tobacco plants. Salinity was induced by adding 150 mM NaCl to the agar medium under the same light conditions, temperature and photoperiod.

Transmission electron microscopy (TEM). Leaf segments (1 cm^2) of the middle sections of leaves from the seedlings were fixed for 24 h in 2.5% glutaraldehyde (Merck, Germany) dissolved in 0.1 M Sorensen's phosphate buffer (pH 7.2) with 1.5% sucrose. Then the samples were washed, post-fixed in 1% OsO_4 (Sigma-Aldrich, USA), dehydrated in ethanol of increased concentrations (30, 50, 70, 96, and 100%) and in propylene oxide (Fluka, Germany). The samples were embedded in mixture of Epon-812 and Araldite (Merck, Germany) according to the standard procedure. For TEM, the embedded samples were sectioned with a diamond knife using an ultramicrotome LKB-V (LKB, Sweden), placed on formvar coated grids and stained with uranyl acetate and lead citrate. The ultrathin sections were examined and photographed with an electron microscope H-500 (Hitachi, Japan). Ultrastructure of mitochondria and peroxisomes in mesophyll cells was studied. For the analysis, fragments of the central cord on the side of the central vein four of the leaf from five independent plants were used. The average cross-sectional area of mitochondria was determined using Cell-A software (Olympus, Japan). At least 200 mitochondria were scored for each treatment.

RESULTS

The role of heterologous expression in plants of the full-length cDNA, encoding mammalian cytochrome P450scc together with its mitochondrial signal sequence (Spivak *et al*., 2009; Shematorova *et al*., 2014), in resistance to ROS-dependent abiotic stresses was shown in early experiments (Shpakovski *et al*., 2017). We studied the alterations of mitochondria and peroxisomes in the course of stress induced by 150 mM NaCl for a period of 14 days on juvenile tobacco plants in *in vitro* culture. Mitochondria and peroxisomes in mesophyll parenchyma cells of control non-transgenic and transgenic tobacco leaves constitutively expressing a full-length cDNA of the *CYP11A1* gene encoding bovine cytochrome P450scc with the signal sequence of mitochondrial targeting were examined. Two homozygous transgenic tobacco lines, TR-2 and TR-7, described previously in Shpakovski *et al*., 2017, were used in the experiments and gave very similar results, which provides additional confirmation of the validity of data obtained.

In cells of tobacco control plants (Fig. 2a, b) affected by salinity a change in shape from rounded to elongated, reduced sectional area, formation of branched mitochondria, as well as the emergence of triangular and diamond-shaped cristae, densification of mitochondrial matrix, and increased density of contrasting membranes and their thickness were observed (Fig. 2c, d). Transgenic plants not treated with NaCl had rounded and elongated mitochondria, twice as small as in control plants (Fig. 3), with dense matrix and tortuous cristae (Fig. 2e, f), as previously described (Shpakovski *et al*., 2017).

Salinity (150 mM NaCl) treatment increased the size of mitochondria of transgenic plants by a factor of 1.5, to a size slightly smaller than mitochondria in NaCl untreated control plants. Salinity treatment also caused matrix decompactisation (Fig. 2g, h; Fig. 3) and development of light zones, presumably containing strands of mitochondrial DNA, in a number of mitochondria (Fig. 2h). Thus, in the course of NaCl exposure, the structural organisation of mitochondria of transgenic plants (Fig. 2g, h) was more comparable to the structural organisation of the mitochondrial compartment of untransformed (non-transgenic) plants (Fig. 2a, b).

Mesophyll cells in control non-transgenic plants treated with saline solution contained round peroxisomes with dense inner content (Fig. 4a, b), and sometimes crystalline inclusions (Fig. 4a) were observed. Under salinity treatment, the number of peroxisomes significantly increased and they all contained crystalline inclusions (Fig. 4c, d). In transgenic plants not treated with NaCl, peroxisomes were

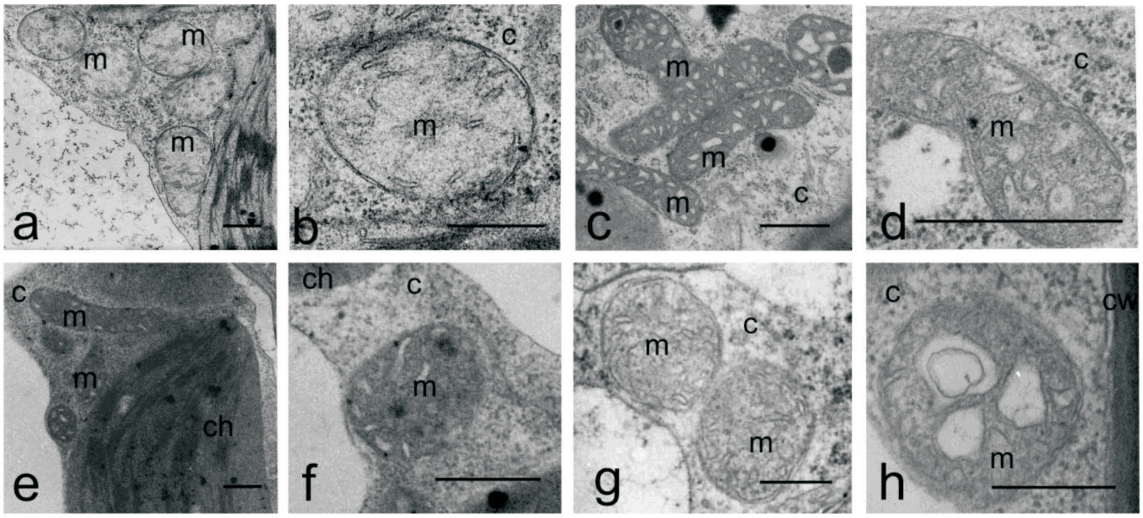

Fig. 2. Mitochondrial ultrastructure of leaf mesophyll cells from seedlings of control non-transgenic tobacco plants (**a, b**) and transgenic tobacco line TR-7 (**e, f**) grown in control medium (MS) and the plants cultivated with 150mM NaCl: wild type tobacco plants (**c, d**) and transgenic tobacco line TR-7 expressing mammalian *CYP11A1* cDNA (**g, h**). Bar – 0.25μm. Abbreviations: m – mitochondria; ch – chloroplast; c – cytoplasm. General view – **a, c, e, g**; separate mitochondria – **b, d, f, h**.

irregularly shaped with sparse internal space and crystalline inclusions (Fig. 4e, f). Under the action of NaCl, the peroxisome shape became round, the inner contents remained loose and multiple damages of outer membranes were noted (Fig. 4g, h).

DISCUSSION

Disturbances in the mitochondrial structure caused by NaCl treatment may result from large accumulation of sodium and/or chloride ions in leaf cells. Differences in the rate of loss of these ions may be caused by the presence of harmful ions that have greater access to mitochondria in plants or by greater sensitivity of mitochondria to excess ion amounts in

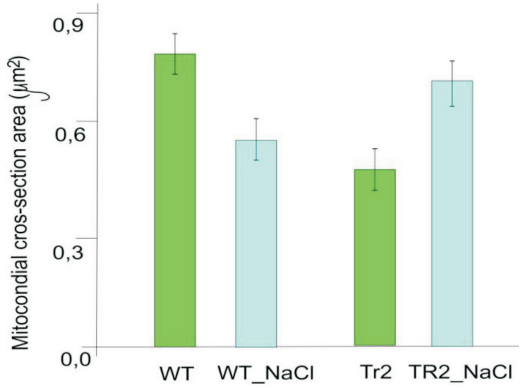

Fig. 3. The average cross-sectional area of mitochondria in cotyledonary mesophyll cells of wild type (WT) and *CYP11A1* transgenic (TR-2) tobacco plants grown with and without 150 mM NaCl.

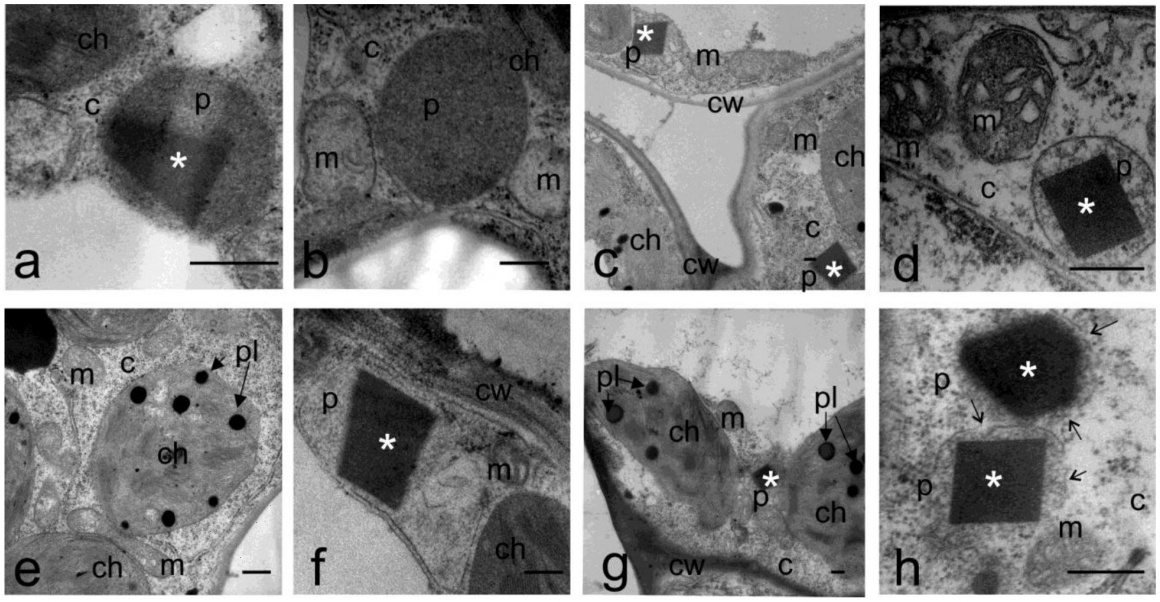

Fig. 4. Peroxisome ultrastructure of leaf mesophyll cells from seedlings of wild type (non-transgenic) tobacco plants (**a, b**) and transgenic tobacco line TR-2 (**e, f**) grown on control medium (MS) and the plants cultivated with 150mM NaCl: wild type tobacco plants (**c, d**) and transgenic tobacco line TR-2 (**g, h**) expressing mammalian *CYP11A1* cDNA. Bar – 0.25 μm. Abbreviations: p – peroxisome; m – mitochondria; ch – chloroplast; pl – plastoglobule; c – cytoplasm; cw – cell wall; * – crystal; thin arrow – membrane rupture.

cells or culture medium. The ultrastructural changes can differ significantly, manifested by increase in size, matrix clarification and decrease in the number of cristae ("swelling"), or in contrast, by reduction in size, contraction of matrix density and the emergence of many cristae having a clear geometric profile and a less discernible thickened membrane (Poljakoff-Mayber, 1975). Mitochondria were observed to be deficient in cristae, swelled and the matrix appeared pale in salt-treated plants as compared to control plants (Rahman *et al*., 2000). Electron micrographs showed an increase in electron optical contrast within the cytosol and in the mitochondria matrix (Koyro, 1997). The observed structural responses all included change in density, size and shape, which indicates an osmotic effect, which was probably not directly related to the action of ions but was caused by increased response in osmolytes, characteristic of plant defense reactions to stressor effects (Vinocur and Altman, 2005). Simultaneously, the change in the structure of membranes and cristae was probably associated with yet another toxic effect of salinity — oxidative stress. Neutralisation of this effect can involve both specific and nonspecific response mechanisms, triggering the expression of stress-regulated genes and changes in the enzyme activity often resulting in the disruption of cellular traffic processes like disruption of cytoskeleton movement (Baranova *et al*., 2016).

Earlier we showed that mesophyll cells of transgenic and non-transgenic tobacco lines differed significantly in the structural organisation of mitochondria and their size. Transgenic tobacco plants expressing *CYP11A1* cDNA (full-length or encoding only the mature cytochrome P450scc) were described in our original patent applications (Kartel *et al*., 2004; 2007), but only transgenic plants bearing the full-length *CYP11A1* cDNA had interesting phenotypes and were further investigated. Mitochondrial localisation of the CYP11A1 (P450scc) mature protein was strongly suggested by results described in one of our previous publications (Shematorova *et al*., 2014: only the mitochondria, and not nuclei, chloroplasts or other cellular compartments, were drastically changed in the transgenic plants studied) and was recently proven by us through the demonstration that apoenzyme (pre-P450ssc) was present both in microsomal and mitochondrial fractions of the transgenic plant cells, but the mature P450ssc was found only in mitochondrial fractions (Shematorova *et al*., 2018).

In the present work we showed that the average mitochondrial cut-off area in cells of *CYP11A1*-transgenic plants was twice less than in the control. In addition, the mitochondria were characterised by electron-dense matrix, as well as a large number of well-formed and enlarged cristae, some of which were parallel to each other, as mentioned also in Shpakovski *et al*., 2017. 150 mM NaCl treatment in the cultivation of *in vitro* culture of the non-transgenic tobacco plants on agar medium led to a decrease in the mitochondria size, increase in their matrix density and emergence of dense triangular and rhomboid cristae. This indicates the formation of a stressful state of mitochondria, but which did not cause a change in their shape and a swelling effect characteristic of NaCl-induced changes in mitochondria in a number of plants (Poljakoff-Mayber, 1975). In the course of salinity treatment, mitochondria of transgenic plants increased in size and formed a structure similar to those observed in wild-type (non-transgenic) plants under native conditions, which indicates an increase in the adaptation of cells of transgenic tobacco plants expressing *CYP11A1* cDNA of animal origin to this stress factor. Our observation of mitochondrial adrenodoxin-like [2Fe–2S] ferredoxins MFDX1 and MFDX2, structural homologues of adrenodoxin reductase MFDR (Shematorova *et al*., 2014), and discovery of membrane receptors of progesterone (Yang *et al*., 2005) and homologues of proteins responsible for delivery of cholesterol in mitochondria of animal cells, allow for the first time to present the general outlines of a second (after brassinosteroids), 'progesterone' system of steroid hormonal regulation, capable of sensing and processing extracellular signals in higher plants (Shpakovski *et al*., 2017).

In our earlier study, we demonstrated the possibility of obtaining a nonspecific mitochondria protection effect in transgenic plants expressing cDNA of Fe-dependent superoxide dismutase 1 gene, equipped in front with the nucleotide sequence encoding signal peptide of chloroplast targeting from the *Rubisco* gene (Baranova *et al*., 2014). An important observation was preservation of only mitochondria from damage and not only the possibility of protecting the non-plastid compartment in principle. This effect can probably be explained by the fact that at present there is no complete and reliable information on precise identification of the transport signal peptides, and possible false deliveries to the non-target compartment may take place (Lee *et al*., 2014; van Wijk *et al.*, 2015). This method of protection against damage based on the principle of "the weakest chain link protection" seems to be the most promising (Gerszberg *et al*., 2017).

The present work on differences in ultrastructural organisation of mitochondria showed the efficiency in use of both the mammalian *CYP11A1* cDNA and the leader peptide signal sequence encoded by it on the formation of targeted protection against the salt-induced damage.

In a study of mesophyll cells under the action of salinity, which is characterised by oxidative stress, it was shown that peroxisomes play an important role (del Rio *et al*., 2002). In view of this, it appears that transgenic plant peroxisomes with cDNA of the mammalian *CYP11A1* gene differed, as anticipated, from the given compartment in the original tobacco both in the normal conditions and under the effect of NaCl (Fig. 4). Various proteins and enzymes associated with peroxisomes are being intensively studied, and it has been revealed that these cellular compartments perform different functions in cells of various tissues and depend on the plant development phase, but many issues remain unresolved (Huang, 2012). The crystalline structures detected in peroxisomes were characterised as catalase and/or associated proteins (Tenberge *et al*., 1997). Changes in the peroxisome structure in *CYP11A1* cDNA expressing trans-

genic plants can be caused by the direct or indirect gene or its product action on cellular metabolism, for example, by a product targeting peculiarities in plant cells, by a gene product effect on regulatory cellular systems or by a specific response to the CYP11A1 (cytochrome P450scc) emergence. The observed changes did not hinder the development of transgenic tobacco plants. Also, deterioration in their growth and development was not observed. In general, the plants were characterised by positive dynamics as compared to the parent ones. Further research will be devoted to a detailed study of this signal sequence and gene exposure at the cellular level and the whole organism under various effects, as well as to the determination of correlation between biochemical parameters and the ultrastructure of this valuable model object. This will allow us to analyse the prospects for use of mammalian signal sequences in plant cells.

Thus, the most important result seems to be the potential use of animal signal sequences for heterologous gene expression in plant cells, which will allow to evaluate the ancient endosymbiotic event results in a new light (Perry *et al*., 2006; Yoshida *et al*., 2017). Until now, this opportunity was clearly demonstrated only in studies performed solely *in vitro* (Luzikov *et al*., 1994).

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REFERENCES

Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J., Hernandez, J. A. (2017). Plant responses to salt stress: Adaptive mechanisms. *Agronomy*, **7** (1), 18.

Baranova, E. N., Christov, N. K., Kurenina, L. V., Khaliluev, M. R., Todorovska, E. G., Smirnova, E. A. (2016). Formation of atypical tubulin structures in plant cells as a nonspecific response to abiotic stress. *Bulg. J. Agric. Sci*., **22** (6), 987–992.

Baranova, E. N., Gulevich, A. A., Maisuryan, A. N., Lavrova, N. V. (2011). Ultrastructure of the cells of tomato transgenic plants with gene *FeSOD* after salinization of nutrient medium. [Баранова, Е. Н., Гулевич, А. А., Майсурян, А. Н., Лаврова, Н. В. Ультраструктурная организация клеток трансгенных растений томата с геном *Fe-SOD* при засолении питательной среды]. *Izv. Timiryazevsk. Skh. Akad*. [*Известия Тимирязевской сельскохозяйственной академии*], No. 1, 90–96 (in Russian).

Baranova, E. N., Kurenina, L. V., Smirnov, A. N., Beloshapkina, O. O., Gulevich, A. A. (2017). Formation of the hypersensitivity response due to the expression of *FeSOD1* gene in tomato when it is inoculated with *Phytophthora infestans*. [Баранова, Е. Н., Куренина, Л. В., Смирнов, А. Н., Белошапкина, О. О., Гулевич, А. А. Формирование реакции сверхчувствительности в результате экспрессии гена *FeSOD1* у томата при инфицировании Phytophthora infestans.] *Russian Agricultural Sciences* [*Российская сельскохозяйственная наука*]. 43 (1), 15–21 (in Russian).

Baranova, E. N., Nodel'man, E. K., Kurenina, L. V., Gulevich, A. A., Baranova, G. B., Bogoutdinova, L. R., Khaliluev, M. R. (2014). Ultrastructural organization of chloroplasts and mitochondria of transgenic tomato plants expressing the *FeSOD1* gene from *Arabidopsis thaliana* (L.) Heynh. under salt stress. *Russian Agric. Sci.*, **40** (6), 426–431.

Corpas, F. J., Barroso, J. B., Sandalio, L. M., Palma, J. M., Lupiáñez, J. A., del Río, L.A. (1999). Peroxisomal NADP-dependent isocitrate dehydrogenase. Characterization and activity regulation during natural senescence. *Plant Physiol*., **121** (3), 921–928.

Foyer, C. H., Noctor, G. (2000). Oxygen processing in photosynthesis: Regulation and signalling. *New Phytol*., **146**, 359–388.

Gerszberg, A., Hnatuszko-Konka, K. (2017). Tomato tolerance to abiotic stress: A review of most often engineered target sequences. *Plant Growth Regul*., **17**, 1–24.

Hammani, K., Giegé, P. (2014). RNA metabolism in plant mitochondria. *Trends Plant Sci*., **19** (6), 380–389.

Huang, A. H. C. (Ed.). *Plant Peroxisomes*. Elsevier, 2012.

Kartel, N. A. Shpakovski, G. V., Spivak, S. G., Brichkova, G. G., Yarmolinsky, D. G., Berdichevets, I. N., Maneshina, T. V. (2004). Recombinant plasmid pGBP450f to obtain transgenic plants and a method of producing transgenic plants with improved productivity and resistance to fungal phytopathogens. [Картель, Н. А., Шпаковский, Г. В., Спивак, С. Г., Бричкова, Г. Г., Ярмолинский, Д. Г., Бердичевец, И. Н., Манешина, Т. В. Рекомбинантная плазмида pGBP450f для получения трансгенных растений и способ получения трансгенных растений табака с повышенной продуктивностью и устойчивостью к грибным фитопатогенам]. Patent of the Russian Federation No. 2237717 [Патент Российской Федерации № 2237717]. Priority from 20.12.2002. Issued 10.10.2004. *Bulletin of Inventions*, No. 10.

Kartel, N. A. Spivak, S. G., Shpakovski, G. V., Maneshina, T. V., Brichkova, G. G., Yarmolinsky, D. G., Berdichevets, I. N. (2007). Genetic engineering construction expressing cDNA of *CYP11A1* gene of animal origin in plants, and a method for producing transgenic plants with increased yield and resistance to phytopathogens. [Генно-инженерная конструкция, экспрессирующая кДНК гена *CYP11A1* животного происхождения в растениях, и метод получения трансгенных растений с повышенной урожайностью и устойчивостью к фитопатогенам]. Patent of the Republic of Belarus No. 9201. [Патент Республики Беларусь № 9201]. Priority from 30.12.2002. Issued 25.01.2007.

Koyro, H. W. (1997). Ultrastructural and physiological changes in root cells of Sorghum plants (*Sorghum bicolor* × *S. sudanensis* cv. Sweet Sioux) induced by NaCl. *J. Exper. Bot*., **48** (3), 693–706.

Lee, J., Kim, D. H., Hwang, I. (2014). Specific targeting of proteins to outer envelope membranes of endosymbiotic organelles, chloroplasts, and mitochondria. *Frontiers Plant Sci*., **5**, 111–116.

Lindemann, P. (2015). Steroidogenesis in plants – Biosynthesis and conversions of progesterone and other pregnane derivatives. *Steroids*, **103**, 145–152.

Luzikov, V. N., Novikova, L. A., Spiridonova, V. A., Isaeva, L. V., Whelan, J., Hugosson M., Glazer E. (1994). Design of heterologous mitochondria: Import of cattle cytochrome P-450scc precursor into plant mitochondria. *Biochemistry (Moscow)*, **59** (7), 1098–1101.

Mittova, V., Guy, M., Tal, M., Volokita, M. (2004). Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii. J. Exper. Bot*., **55** (399), 1105–1113.

Møller, I. M. (2001). Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **52**, 561–591.

Murashige, T., Skoog, F. A. (1962). Revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.*, **15** (3), 473–497.

Pauli, G. F., Friesen, J. B., Gödecke, T., Farnsworth, N. R., Glodny, B. (2010). Occurrence of progesterone and related animal steroids in two higher plants. *J. Nat. Prod*., **73** (3), 338–345.

Perry, A. J., Hulett, J. M., Likić V. A., Lithgow T., Gooley P. R. (2006). Convergent evolution of receptors for protein import into mitochondria. *Curr. Biol*., **16** (3), 221–229.

Poljakoff-Mayber, A. (1975). Morphological and anatomical changes in plants as a response to salt stress. In: Poljakoff-Mayber, A., Gale, J. (eds.). *Plants in Saline Environments*. Springer-Verlag, Berlin, pp. 97–117.

Rahman, S., Matsumuro, T., Miyake H., Takeoka Y. (2000). Salinity-induced ultrastructural alterations in leaf cells of rice (*Oryza sativa* L.). *Plant Prod. Sci*., **3** (4), 422–429.

Rejeb, I. B., Pastor V., Mauch-Mani B. (2014). Plant responses to simultaneous biotic and abiotic stress: Molecular mechanisms. *Plants*, **3** (4), 458–475.

del Rio, L. A., Corpas, F. J., Sandalio, L. M., Palma, J. M., Gomez, M., Barroso, J. B. (2002). Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. *J. Exper. Bot*., **53**, 1255–1272.

Saxena, B., Shukla, K., Giri, B. (2017). Arbuscular mycorrhizal fungi and tolerance of salt stress in plants. In: Wu, Qiang-Sheng (Ed.). *Arbuscular Mycorrhizas and Stress Tolerance of Plants*. Springer, Singapore, pp. 67–97.

Serenko, E. K., Baranova, E. N., Balakhnina, T. I., Kurenina, L. V., Gulevich, A. A., Kosobruhov, A. A., Polyakov, V. Y. (2011). Structural organization of chloroplast of tomato plants *Solanum lycopersicum* transformed by Fe-containing superoxide dismutase. *Biochemistry (Moscow). Supplemental Series A: Membrane and Cell Biology*, **5** (2), 177–184.

Shematorova, E. K., Slovokhotov, I. Yu., Baranova, E. N., Khaliluev, M. R., Babak, O. G., Klykov, V. N., Shpakovski, D. G., Spivak, S. G., Shpakovski, G. V. The role of organelles in the functioning of steroid hormonal systems in animals and higher plants. [Шематорова, Е. К., Словохотов, И. Ю., Баранова, Е. Н., Халилуев, М. Р., Бабак, О. Г., Клыков, В. Н., Шпаковский, Д. Г., Спивак, С. Г., Шпаковский, Г. В. Роль органелл в функционировании стероидных гормональных систем у животных и высших растений]. Proceedings of the International Conference "Mechanisms of regulation of eukaryotic cell organelles functions" [Механизмы регуляции функций органелл эукариотической клетки], Irkutsk, 22–24 May 2018), pp. 155–157 (in Russian).

Shematorova, E. K., Slovokhotov, I. Y., Khaliluev, M. R., Berdichevets, I. N., Baranova, E. N., Babak, O. G., Shpakovski, D. G., Spivak, S. G., Shpakovski, G. V. (2014). Mitochondria as a possible place for initial stages of steroid biosynthesis in plants. [Шематорова, Е. К., Словохотов, И. Ю., Халилуев, М. Р., Бердичевец, И. Н., Баранова, Е. Н., Бабак, О. Г., Шпаковский, Д. Г., Спивак, С. Г., Шпаковский, Г.В. Митохондрии как возможное место инициации синтеза стероидных гормонов в растениях]. *J. Stress Physiol. Biochem*. [*Журнал стресс-физиологии и биохимии*], **10** (4), 85–97 (in Russian).

Shpakovski, G. V., Spivak, S. G., Berdichevets, I. N., Babak, O. G., Kubrak, S. V., Kilchevsky, A. V., Aralov, A. V., Slovokhotov, I. Yu., Shpakovski, D. G., Baranova, E. N., Khaliluev, M. R., Shematorova, E. K. (2017). A key enzyme of animal steroidogenesis can function in plants enhancing their immunity and accelerating the processes of growth and development. *BMC Plant Biol*., **17** (Suppl 1): 189, pp. 120–131.

Simerský, R., Novák, O., Morris, D. A., Pouzar, V., Strnad, M. (2009). Identification and quantification of several mammalian steroid hormones in plants by UPLC-MS/MS. *J. Plant Growth Regul*., **28** (2), 125–136.

Spivak, S. G., Berdichevets, I. N., Litvinovskaya, R. P., Drach, S. V., Kartel, N. A., Shpakovski, G. V. (2010). Some peculiarities of steroid metabolism in transgenic *Nicotiana tabacum* plants bearing the *CYP11A1* cDNA of cytochrome P450scc from the bovine adrenal cortex. *Russ. J. Bioorg. Chem*., **36** (2), 224–232.

Spivak, S. G., Berdichevets, I. N., Yarmolinsky, D. G., Maneshina, T. V., Shpakovski, G. V., Kartel, N. A. (2009). Construction and characteristics of transgenic tobacco *Nicotiana tabacum* L. plants expressing *CYP11A1* cDNA encoding cytochrome P450scc. *Russ. J. Gen*., **45** (9), 1067–1073.

Tenberge, K. B., Ruholl, C., Heinze, M., Eising, R. (1997). Purification and immuno-electron microscopical characterization of crystalline inclusions from plant peroxisomes. *Protoplasma*, **196** (3), 142–154.

Verma, V., Ravindran, P., Kumar, P. P. (2016). Plant hormone-mediated regulation of stress responses. *BMC Plant Biol*., **16** (1), 86.

Vinocur, B., Altman, A. (2005). Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. *Curr. Opin. Biotechnol.*, **16** (2), 123–132.

Wang, W., Vinocur, B., Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, **218** (1), 1–14.

van Wijk, K. J. (2015). Protein maturation and proteolysis in plant plastids, mitochondria, and peroxisomes. *Annu. Rev. Plant Biol*., **66**, 75–111.

Yang, X. H., Xu, Z. H., Xue, H. W. (2005). Arabidopsis membrane steroid binding protein 1 is involved in inhibition of cell elongation. *Plant Cell*, **17** (1), 116–131.

Ylstra, B., Touraev, A., Brinkmann, A. O., Heberle-Bors, E., Tunen, A. (1995). Steroid hormones stimulate germination and tube growth of *in vitro* matured tobacco pollen. *Plant Physiol*., **107** (2), 639–643.

Yoshida, T., Furihata, H. Y., Kawabe, A. (2017). Analysis of nuclear mitochondrial DNAs and factors affecting patterns of integration in plant species. *Genes Genetic Syste*., **92** (1), 27–33.

Zlobin, I. E., Kartashov A. V., Shpakovski, G. V. (2017). Different roles of glutathione in copper and zinc chelation in *Brassica napus* roots. Plant *Physiol. Biochem*., **118** (9), 333–341.

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CYP11A1 cDNS EKSPRESĒJOŠO TRANSGĒNO TABAKAS AUGU MEZOFĪLO ŠŪNU MITOHONDRIJU MĒRĶĒTA AIZSARDZĪBA NO NaCl STRESA INDUCĒTIEM BOJĀJUMIEM

Zīdītāju *CYP11A1* cDNS ekspresija augiem paaugstina to izturību pret abiotiskiem un biotiskiem stresiem. Darbā pētītas juvenīlo tabakas augu mitohondriju strukturālās izmaiņas NaCl stresa ietekmē mezofīlās šunās, kuras ekspresē *CYP11A1* cDNS. Zīdītāju CYP11A1 proteīns var veidot mērķētu mitohondriju aizsardzību no sāls inducētā stresa.