biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2021) 65: 111-117 DOI: 10.32615/bp.2020.139

Tolerance of *Arabidopsis thaliana* plants overexpressing grapevine *VaSTS1* or *VaSTS7* genes to cold, heat, drought, salinity, and ultraviolet irradiation

Z.V. OGNEVA[1], O.A. ALEYNOVA[1], A.R. SUPRUN[1,2], Y.A. KARETIN[3], A.S. DUBROVINA[1],
and K.V. KISELEV [1,*]

[1] *Laboratory of Biotechnology, Federal Scientific Center of the East Asia Terrestrial Biodiversity, FEB RAS, Vladivostok, 690022, Russia,*
[2] *Department of Biodiversity, The School of Natural Sciences, Far Eastern Federal University, Vladivostok, Russia,*
[3] *Laboratory of Embryology, National Scientific Center of Marine Biology, FEB RAS, Vladivostok 690059, Russia*

[*]Corresponding author: E-mail: kiselev@biosoil.ru

Abstract

Stilbene synthases (STS) are plant enzymes that are responsible for the biosynthesis of stilbenes, which are plant phenolic compounds with valuable biological properties. Stilbenes also play important roles in plant tolerance to biotic and abiotic stresses. Therefore, plants that overexpress *STS* genes can be more resistant to various stresses. This paper investigated the effects of *STS* gene overexpression in *Arabidopsis thaliana* (L.) Heynh. Columbia-0 plants on stilbene content and tolerance to the following abiotic stresses: low and high temperatures, salinity, drought, and ultraviolet irradiation (UV-B and UV-C). We used *VaSTS1* and *VaSTS7* genes from grapevine (*Vitis amurensis* Rupr.) expressed under the double cauliflower mosaic virus 35S (CaMV35S) promoter. This study firstly demonstrated that overexpression of the *VaSTS1* and *VaSTS7* genes in *A. thaliana* plants considerably increased plant tolerance to UV-B and UV-C, while the tolerance to the low and high temperatures, salinity, and drought was not affected. We showed that the highest *trans*-piceid and *trans*-resveratrol total content was in ST1 *A. thaliana* plants that overexpressed the *VaSTS1* gene in the range 8.28 - 22.66 µg g^{-1}(f.m.). ST7 plants that overexpressed the *VaSTS7* gene showed only *trans*-resveratrol at 0.02 - 0.08 µg g^{-1}(f.m). Stilbene content and UV tolerance in transgenic *A. thaliana* plants correlated with *STS* transgene expression. *STS* expression, UV tolerance, and stilbene content was higher in *VaSTS1* transgenic plants compared with that in *VaSTS7* transgenic plants.

Keywords: glucosyltransferase, peroxidase, piceid, resveratrol, stilbene synthase, viniferin.

Introduction

Stilbenes are natural phenolic compounds that occur in several unrelated plant families, including *Pinaceae* (spruce, pine), *Fabaceae* (false acacia, peanut), *Polygonaceae* (knotweed, rhubarb), and *Vitaceae* (grapevine) (Dubrovina and Kiselev 2017). Stilbenes are biologically active (Kiselev 2011, Shankar *et al.* 2011, Suwalsky *et al.* 2015), and they also play a positive role in plant tolerance to abiotic and biotic stresses. Stilbenes are involved in constitutive and inducible plant defense responses as phytoalexins and have also attracted interest due to their protective properties against plant fungal pathogens, nematodes, and herbivores (Chong *et al.* 2009, Jeandet *et al.* 2010, Laavola *et al.* 2015, Weiskirchen and Weiskirchen 2016). Stilbenes directly affect pathogens by inhibiting their growth and protect the plant host cells from excessive destructive oxidative stress, which always appears under pathogen attack.

Stilbenes are synthesized *via* the phenylpropanoid

Received 5 July 2020, *last revision* 17 September 2020, *accepted* 18 September 2020.

Abbreviations: f.m. - fresh mass; MS - Murashige and Skoog medium; STS - stilbene synthase; UV - ultraviolet.

Acknowledgements: This study was supported by a grant from the Russian Foundation for Basic Research (19-04-00063-a and 19-316-90004). The 19-04-00063-a supported obtaining *VaSTS1* or *VaSTS7* overexpressed *Arabidopsis* plants, RNA, DNA analysis, and qRT-PCR. The 19-316-90004 supported experiments on *A. thaliana* plants stress tolerance.

Conflict of interest: The authors declare that they have no conflict of interest.

pathway (Langcake and Pryce 1977), where stilbene synthase (STS, EC 2.3.1.95) directly catalyzes the resveratrol (monomeric stilbene) formation by condensing three molecules of malonyl-CoA and one molecule of p-coumaroyl-CoA to form the stilbene backbone. It has been shown that some stress conditions caused considerable induction in stilbene biosynthesis in plants (*e.g.* grapes), which indicates the stilbene participation in the defense against the stress conditions (reviewed in Dubrovina and Kiselev 2017).

There are several approaches to establish plants more tolerant to abiotic stresses. One of them is the obtaining of transgenic plants, overexpressing different *STS* genes. For example, He *et al.* (2018) transformed *Nicotiana benthamiana* using the *Polygonum cuspidatum PcSTS1* gene, and they showed that the transgenic plants accumulate resveratrol [21.1 μg g^{-1}(f.m.)]. In addition to an increase in resistance to the pathogen *Monilinia fructicola*, the transgenic plants exhibite improved tolerance to salt and osmotic stresses (He *et al.* 2018). Overexpressing the *SSvin* gene from grapevine in kiwifruit *Actinidia deliciosa* lead to the piceid production in the transgenic kiwifruit [182 μg g^{-1}(f.m.)], but those kiwifruit plants are not resistant against the gray-mold disease caused by *Botrytis cinerea* (Kobayashi *et al.* 2000).

In 2004, Giorcelli *et al.* overexpressed the *Vitis vinifera StSy* gene in *Populus alba*. Two new compounds were detected in transgenic poplar plants: the piceid *trans*- and *cis*-isomers together up to 615 μg g^{-1}(leaf f.m.). However, poplar transgenic plants are not resistant against the pathogen *Melampsora pulcherrima*, which causes rust diseases (Giorcelli *et al.* 2004). Another paper (Yu *et al.* 2006) described overexpression of the *Sorghum bicolor SbSTS1* gene in *Arabidopsis thaliana tt4* mutants, which are defective in chalcone synthase activity. Firstly, the authors found *cis*-piceid up to 580 μg g^{-1}(f.m.) in the *Arabidopsis* transgenic lines (Yu *et al.* 2006). Later, three additional resveratrol-related metabolites are found in the transgenic *Arabidopsis* plants, including a resveratrol diglucoside up to 2.8 μg g^{-1}(f.m.) and *trans*- and *cis*-resveratrol acetylhexosides up to 0.07 and 10.4 μg g^{-1}(f.m.), respectively (Lo *et al.* 2007). Liu *et al.* (2011) also used *A. thaliana* for experiments, but they overexpressed the *P. cuspidatum PcRS* gene. The transgenic *Arabidopsis* plants produced a new compound in both the leaves and seeds, which was identified as *trans*-piceid up to 15.2 - 183.7 μg g^{-1}(f.m.). Moreover, the transgenic *Arabidopsis* plants show enhanced resistance to its fungal pathogen *Colletotrichum higginsianum*, modified seed coat pigmentation, and a significant reduction in anthocyanin content (Liu *et al.* 2011).

There are some data regarding the content of stilbenes and the pathogen resistance of transgenic plants. It has been shown previously that stilbene content considerably increased after UV irradiation in grapevine (Duan *et al.* 2015, Tyunin and Kiselev 2016, Kiselev *et al.* 2019b), but no one analyzed the tolerance of *STS* transgenic plants to the UV irradiation, cold, and heat stresses. Therefore, we decided to obtain *STS* transgenic *Arabidopsis* plants and study the content of stilbenes and their tolerance for abiotic stresses (UV radiation, cold, heat, drought, and salt stresses). We used two *STS* genes from wild-growing grapevine that were not previously used in other papers for plant transformation.

Materials and methods

Plant cultivation and stress induction: Plants of *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia-0 (stored by our laboratory) were grown in pots filled with commercially available rich soil in a controlled environmental chamber (*Sanyo MLR-352, Panasonic*, Osaka, Japan) kept on a 16-h photoperiod, an irradiance of ~120 μmol m^{-2} s^{-1}, and a temperature of 22 ºC. Wild-type and transgenic sterilized seeds were germinated on 1/2 Murashige and Skoog medium (1962; MS), pH 5.6, solidified with 0.8 % (m/v) agar. Then, the seedlings grown on the MS medium for 7 - 8 d were transferred to commercially available soil. The plants were treated with different stresses (cold, drought, salt, and heat stresses) as described in Dubrovina *et al.* (2017). Briefly, after planting in the soil the plants were subjected to drought by culturing without additional irrigation for 5 weeks and then re-watered. For salt stress treatments, the transferred seedlings were cultivated without additional irrigation for 2 weeks and then the plants were irrigated with 350 mM NaCl solution. One week after irrigation with NaCl the pots were placed in fresh water for 3 - 4 h to leach the salt from the soil. For cold stress assay, normally cultured *A. thaliana* plants (3-week-old) were stressed at -10 °C in freezer for 1 h and then cultured at 8 °C for 2 h and after that transferred to normal conditions. For heat stress assays, normally cultured plants (3-week-old) were stressed at 45 °C in a controlled incubator for 3 h. The survival rates were determined as the number of visibly green plants 3 d after re-watering (drought), 1 week after heat and cold stress treatments and 1 week after salt leaching (salt stress). Two pots of plants (10 seedlings per pot) were grown for each transgenic line and each treatment. The experiments were repeated at least eight times.

We did not test the effect of UV rradiation on the survival of *Arabidopsis* plants, because the treatment killed the overground part of plants. However, all the plants produced new leaves after a week, because the underground part of all plants remained alive. Therefore, we analyzed leaves viability of the irradiated plants. Four-week-old Arabidopsis plants were exposed to UV-B (312 nm) or UV-C (254 nm) using a UV lamp *VL-215.MC* provided by *Vilber Lourmat Company* (Marne-la-Vallee, France). The plants were irradiated for 10 min at a distance of 15 cm above pots and peak output being 254 or 312 nm as described (Tyunin and Kiselev 2016). According to the manufacturer's manual, we used 1800 μW cm^{-2} of UV-B and 930 μW cm^{-2} of UV-C irradiation. The leaf survival rates were determined as the number of visibly green leaves 1 d after the end of stress .

Obtaining *VaSTS1* or *VaSTS7* overexpressing *Arabidopsis* plants: To create transgenic *Arabidopsis* plant lines overexpressing the *VaSTS1* (Genebank GQ167204) or *VaSTS7* (EU659868) genes from *Vitis amurensis*, we used pZP-RCS2-(*VaSTS1* or *VaSTS7*)-*npt*II plasmid construction (Aleynova *et al.* 2016, Kiselev and Aleynova 2016). The constructs pZP-RCS2-(*VaSTS1* or *VaSTS7*)-*npt*II or empty vector pZP-RCS2-*npt*II were introduced into the *Agrobacterium tumefaciens* strain GV3101::pMP90 and transformed by floral dip method as described previously into wild-type *A. thaliana* Col-0 plants (Zhang *et al.* 2006) for generating the *VaSTS1*- or *VaSTS7*-overexpressing lines.

Transgenic plants were selected by kanamycin resistance on half-strength MS medium supplemented with 50 mg dm^{-3} kanamycin and confirmed by PCR using the primers for *VaSTS1*, *VaSTS7*, and *npt*II genes listed in the Table 1 Suppl. The PCR products were verified by DNA sequencing (*ABI 3130*, *Applied Biosystems*, Foster City, CA, USA). Three representative independent T_4 homozygous lines with single copy transgene insertion (ST1-1, ST1-2, ST1-3 and ST7-1, ST7-2, ST7-3) with different mRNA amounts of *VaSTS1* or *VaSTS7* genes were chosen for detailed analyses.

RNA, DNA analysis, and real-time quantitative PCR (qPCR): Total RNA isolation from plant leaves was performed using the cetyltrimethylammonium bromide-based extraction (Kiselev *et al.* 2013, Ogneva *et al.* 2019). Complementary DNA was synthesized using 1.5 µg of total RNA by the RNA PCR kit (*Silex M*, Moscow, Russia) as described (Kiselev *et al.* 2007). The real-time qPCRs were performed using a *HS* polymerase and *50X SYBR Green I* (*Evrogen*, Moscow, Russia) in a thermocycler supplied with real-time PCR detection system (*DNA Technology*, Moscow, Russia). Expression was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001), where the value 1 in the relative mRNA calculation in each qPCR reaction was assigned for transgenic plants overexpressing only selective marker *npt*II gene (KA0). *AtActin2* (GB acc. No. NM_112764) and *AtGAPDH* (GB acc. No. NM_111283) genes were used as endogenous controls to normalize variance in the quality and the amount of cDNA of *A. thaliana* used in each real-time qPCR experiment (Czechowski *et al.* 2005, Dubrovina *et al.* 2020).

The primers used for analyzing the *VaSTS1* and *VaSTS7* transcriptions in the transgenic *Arabidopsis* plants (Table 1 Suppl.) were designed to the 3' end of the protein coding region of the *VaSTS1* or *VaSTS7* mRNA (the cloning *VaSTS1* and *VaSTS7* genes have the same nucleotide sequence in the end of the protein coding region) and to the CaMV 35S terminator in the pSAT1 vector. Real-time qPCR data were obtained from at least three independent experiments and are averages of eight technical replicates for each independent experiment (Dubrovina *et al.* 2020).

Total DNA was purified from 20 mg of dried *A. thaliana* tissues (mixed rosette leaves) using the *EZ DNA methylation-Gold* kit (*Zymo Research*, Irvine, CA, USA) from the eight-week-old *A. thaliana* plants as described (Kiselev *et al.* 2015). Bisulfite sequencing was performed as stated in Ogneva *et al.* (2016). After DNA conversion, 352-bp *npt*II fragments were amplified using primers listed in Table 1 Suppl. We sequenced 16 clones for each DNA region from the 2 biological replicates (8 clones per each individual plant).

Analytical chromatography: All solvents were of high performance liquid chromatography (HPLC) grade. Analytical standards: *trans*-resveratrol and *trans*-piceid were obtained from *Sigma-Aldrich* (St. Louis, MO, USA); ε-viniferin was obtained from *Panreac AppliChem* (Darmstadt, Germany); *trans*-piceatannol was obtained from *Enzo Life Sciences* (Farmingdale, NY, USA). The aboveground part of 35-d-old plants was dried using hot air flow (50 °C for 2 h), weighted and used for stilbene detection. Samples containing 100 mg of dried tissues were extracted with 2 cm^3 of 96 % (v/v) ethanol at 60 °C for 2 h. Stilbene content was analyzed at least three times. The HPLC was carried out using an *LC-20* analytical HPLC system (*Shimadzu*, Kyoto, Japan), equipped with a *SPD-M20A* photodiode array detector, *LC-20ADXR* pump, *Shim-pack XR-ODS II* column and SIL-20ACXR auto sampler as described (Aleynova *et al.* 2016).

Analysis for statistical differences and correlation: The statistical analysis for significant differences and correlation in experiments was carried out using the *Statistica 10.0* program (*StatSoft*, Boston, USA). The data are presented as means ± standard errors (SEs) and were tested by paired Student's *t*-test.

Results and discussion

After selection on kanamycin, we obtained three lines of *Arabidopsis* homozygous T3 plants that overexpressed *VaSTS1* (ST1 lines) gene and three lines that overexpressed *VaSTS7* gene (ST7 lines). All lines were obtained as a result of independent transformations. Next, we analyzed the expression of transferred *STS* transgenes. We used

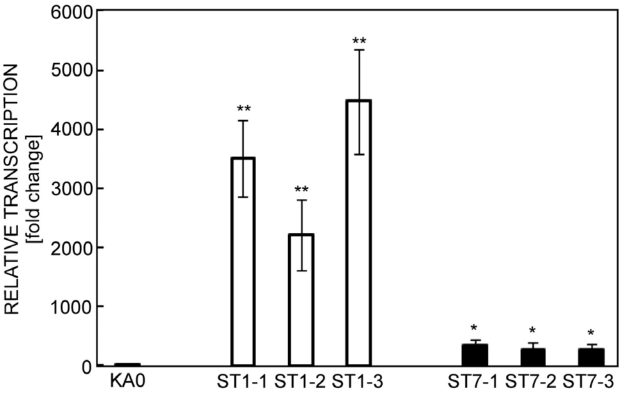

Fig. 1. Quantification the *VaSTS1* or *VaSTS7* gene expressions in the transgenic *Arabidopsis* plants. KA0 - transgenic plants overexpressing only selective marker *npt*II gene, ST1-1, -2, or -3 - lines overexpressing *VaSTS1* gene, ST7-1, -2, or -3 - lines overexpressing *VaSTS7* gene. Means ± SE, $n = 8$; * - $P < 0.05$, ** - $P < 0.01$ *versus* value of fluorescence in KA0 plants (paired Student's *t*-test).

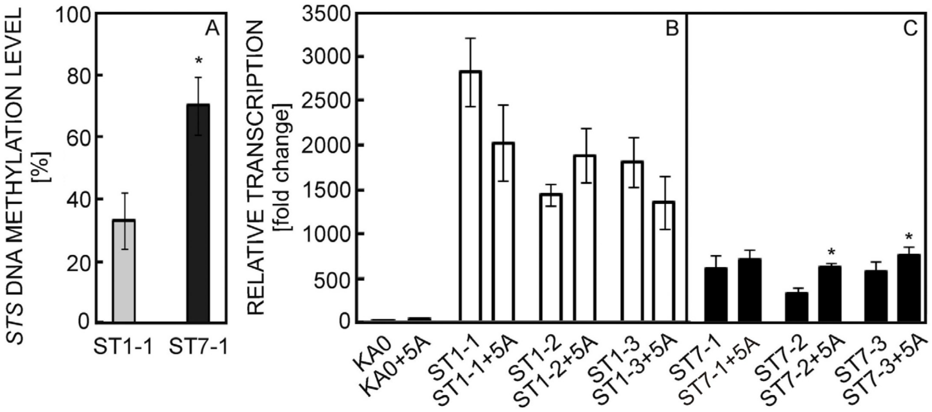

Fig. 2. Quantification the *VaSTS* methylation in the transgenic *Arabidopsis* plants (*A*) and influence of the 5-azacytidine (5A) treatment on the *VaSTS1* (*B*) and *VaSTS7* (*C*) gene expression. KA0 - transgenic plants overexpressing only selective marker *npt*II gene; ST1-1, -2, -3 - plant lines overexpressing *VaSTS1* gene; ST7-1, -2, -3 - plant lines overexpressing *VaSTS7* gene. Means ± SEs, $n = 8$;. * - $P < 0.05$ *versus* values of DNA methylation in STS1-1 plants (*A*) or *VaSTS* expression in 5A untreated plants (*B*) (paired Student's *t*-test).

the same primers for the analysis of *VaSTS1* and *VaSTS7* genes, and we compared *STS* gene expression between them and with KA0 transgenic *Arabidopsis* plants that overexpressed only *npt*II gene, a selective marker for transgene plant selection (Fig. 1).

In all transgenic plants, the *STS* gene amplification was significantly higher compared with the background level in control KA0 *Arabidopsis* plants (Fig. 1). Thus, all *STS* transgenic *Arabidopsis* plants expressed *STS* genes. Moreover, we showed that *VaSTS1* gene expression in all ST1 lines was 6.5 - 13.3 times higher than the *VaSTS7* gene expression in all ST7 lines (Fig. 1). These results were unexpected because *VaSTS1* and *VaSTS7* genes are under the control of the same strong constitutive promoter cauliflower mosaic virus 35S and therefore might have approximately the same expression.

Transgenes in the plants are known to be inhibited by cytosine DNA methylation gene silencing (Rajeevkumar *et al.* 2015). Perhaps in our case, *STS* gene sequences undergo different levels of methylation. Thus, we analyzed the cytosine methylation of the *VaSTS1* and *VaSTS7* gene sequence in the ST1-1 and ST7-1 lines using bisulfite sequencing (Rajeevkumar *et al.* 2015). We demonstrated that the cytosine methylation of the 3′-end of the protein coding region of the *VaSTS1* gene in line ST1-1 was 33.3 ± 9.0 %, while methylation of the *VaSTS7* gene in the ST7-1 line was 2.1-times higher (70.1 ± 9.4 %, Fig. 2*A*). Thus, the *VaSTS7* gene was hypermethylated and this is the reason for the low *VaSTS7* gene expression in transgenic ST7 *Arabidopsis* lines.

5-Azacytidine (5A), a chemical analogue of the nucleoside cytidine, is an inhibitor of DNA methylation (Kiselev *et al.* 2019a). We showed that treatment with 5A increased the *VaSTS1* gene expression only in ST1-2 line by 1.3 times (Fig. 2*B*). However, this elevation of the *VaSTS1* gene expression was not significant. At the same time 5A increased hypermethylated *VaSTS7* gene expression by 1.2 - 1.9 times in all the ST7 lines of the transgenic *Arabidopsis* plants (Fig. 2*C*). Thus, 5A decreased total DNA methylation, which leads to increased expression of the more methylated *VaSTS7* genes (Fig. 2*C*). Expression of the less methylated *VaSTS1* gene after 5A treatment did not change significantly (Fig. 2*B*).

Then, we found that no stilbenes were detected using HPLC in KA0 *A. thaliana* plants that did not express grapevine *STS* genes (Fig. 1*A* Suppl., Table 1), while transgenic *A. thaliana* plants overexpressing the *VaSTS1* gene produced two stilbenes: *trans*-piceid and *trans*-resveratrol (Fig. 1*B* Suppl., Table 1). Plants overexpressing the *VaSTS7* gene produced only *trans*-resveratrol (Table 1).The highest *trans*-piceid and *trans*-resveratrol content was in *A. thaliana* plants that overexpressed the *VaSTS1* gene: 8.28 - 22.66 μg g^{-1}(f.m.) (Table 1). The highest stilbene content among the *VaSTS1* transgenic plant lines was in ST1-3, while the lowest was in ST1-2 (Table 1). This stilbene content positively correlated with *VaSTS1* gene expression ($r = 0.96$). Total stilbene content in ST7 plants overexpressing the *VaSTS7* gene was in 103 - 1133 times lower than the stilbene content in the *VaSTS1* transgenic plant lines, we found only *trans*-resveratrol: 0.02 - 0.08 μg g^{-1}(f.m.) (Table 1) and this result also correlated ($r = 0.98$) with low *VaSTS7* transgene expression.

There are several papers in which different transgenic plants overexpressing different *STS* genes have been obtained. Thus, analyzing the available papers, we have shown that the content of stilbenes in our ST1-3 *Arabidopsis* plants (highest stilbene content in our experiments) was 50.4-times higher compared with *Ziziphus jujuba*. [0.45 μg g^{-1}(f.m.)] that overexpressed the *P. cuspidatum PcPKS5* gene (Luo *et al.* 2015). However, the content of stilbenes in the transgenic white poplar overexpressing the *V. vinifera StSy* gene was 27.1 times higher [up to 615 μg g^{-1}(leaf f.m.)] of *trans*- and *cis*-isomers of piceid) compared with stilbene content in ST1-3 *Arabidopsis* plants (Giorcelli *et al.* 2004). There were also no stilbenes in transgenic strawberries that overexpressed *NS-Vitis3* gene encoding *STS* from *Vitis riparia* (Hanhineva *et al.* 2009).

Unfortunately, stilbenes in transgenic *Arabidopsis* plants overexpressing *STS* genes were not analyzed (Liu *et al.* 2011) or *STS* genes were transferred to *Arabidopsis*

Table 1. Content of *trans*-piceid and *trans*-resveratrol in the KA0, ST1-1, 2, 3, ST7-1, 2, 3 *Arabidopsis* plants, overexpressing the *VaSTS1* or *VaSTS7* genes. The plant samples were harvested from the 35 d-old plants. Means ± SEs, $n = 8$; * - $P < 0.05$, ** - $P < 0.01$ *versus* values in the empty vector-transformed KA0 plants.

Cell lines	*Trans*-piceid [μg g^{-1}(f.m.)]	*Trans*-resveratrol [μg g^{-1}(f.m.)]	Total stilbenes [μg g^{-1}(f.m.)]
KA0	0	0	0
ST1-1 (*VaSTS1*)	12.42 ± 4.11*	0.32 ± 0.23	12.73 ± 4.34*
ST1-2 (*VaSTS1*)	8.01 ± 2.02**	0.28 ± 0.19	8.28 ± 2.19*
ST1-3 (*VaSTS1*)	21.43 ± 4.98**	1.24 ± 0.55*	22.66 ± 5.56**
ST7-1 (*VaSTS7*)	0	0.08 ± 0.03	0.08 ± 0.03
ST7-2 (*VaSTS7*)	0	0.02 ± 0.02	0.02 ± 0.02
ST7-3 (*VaSTS7*)	0	0.02 ± 0.02	0.02 ± 0.02

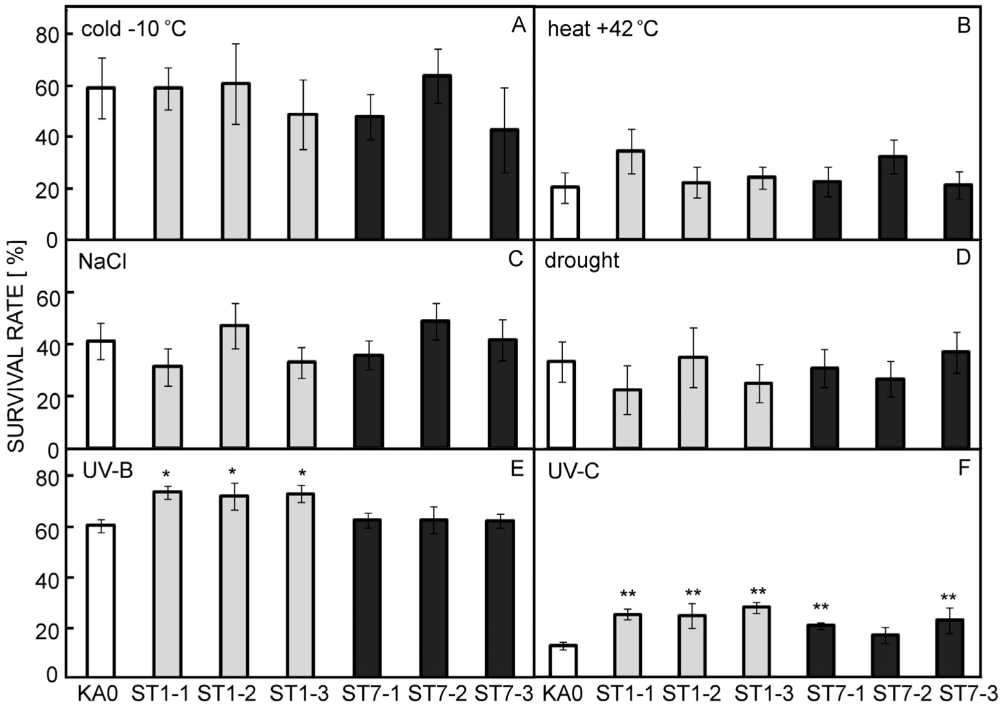

Fig. 3. Survival rates of *Arabidopsis* transgenic plants (*A-D*) and leaves (*E,F*) after different stresses: cold (*A*), heat (*B*), salinity (*C*), drought (*D*), ultraviolet B irradiation (UV-B, *E*), and ultraviolet C irradiation (UV-C, *F*). KA0 - plants overexpressing only selective marker *npt*II gene; ST1-1, -2, or -3 - lines overexpressing *VaSTS1* gene; ST7-1, -2, or -3 - lines overexpressing *VaSTS7* gene. Means ± SEs, $n = 160$; * - $P < 0.05$, ** - $P < 0.01$ *versus* values of plant or leaves survival rate in KA0 plants (paired Student's *t*-test).

tt4 mutants plants, which could not make flavonoids (Buer and Muday 2004). Flavonoids are competitors of stilbenes in the biosynthesis pathway because flavonoids and stilbenes use the same precursors (Shumakova *et al.* 2011). Therefore, precursors of phenolic compounds were used in the stilbene biosynthesis. In those plants, the content of stilbenes reaches 600 μg g^{-1}(f.m.) (*cis*-piceid, resveratrol diglucoside, and *trans*- and *cis*-resveratrol acetylhexosides), which is one of the highest contents, and this may be because there is an excess of the stilbene precursors in these plants (Yu *et al.* 2006, Lo *et al.* 2007). Thus, our ST1 lines for the production of stilbenes were closer to the average level in transgenic plants, and our ST7 lines had one of the lowest stilbene content.

Transgenic *Arabidopsis* plants were exposed to the main abiotic stresses: low and high temperatures, salinity, drought, and UV radiation (UV-B, 312 nm and UV-C, 254 nm). Our results showed that overexpression of the *VaSTS1* and *VaSTS7* gene did not increase tolerance to salinity, drought, and low temperatures (Fig. 3*A*,*C*,*D*). We showed that viability of the all *STS* overexpressed plants after heat treatment was on 1 - 14 % higher compared with KA0 plants, but this enhancement was not statistically significant (Fig. 3*B*).

We detected increased tolerance to UV radiation: *VaSTS1* gene overexpression elevated the quantity of green leaves remained after UV-B treatment (1.2 - 1.3 times) (Fig. 3*E*, Fig. 4). This enhancement was statistically significant for all *VaSTS1*-transgenic *Arabidopsis* plants (Fig. 3*E*). *VaSTS7* gene overexpression also slightly

Fig. 4. Survival rates of *Arabidopsis* leaves after UV-B and UV-C irradiation. Four-week-old plants were exposed to UV-B (312 nm) or UV-C (254 nm) radiation (1800 µW cm^{-2} of UV-B and 930 µW cm^{-2} of UV-C). The leaf survival rates were determined as the number of visibly green leaves 1 d after the irradiation.

increased the viability of the UV-B irradiated plants, but this enhancement was not statistically significant (Fig. 3*E*).

Similar results were obtained when UV-C was applied, but the negative impact on the survival of the leaves under the same conditions (time, distance to plants from the UV-C lamp) was much stronger (Fig. 3*F*, Fig. 4), although the UV-B radiation intensity was almost 2 times greater (1 800 µW cm^{-2} of UV-B *versus* 930 µW cm^{-2} of UV-C). This confirms earlier findings that UV-C exposure is more damaging for plants than UV-B exposure (Nawkar *et al.* 2013). Under more severe conditions of UV-C irradiation, the protective effect of the *VaSTS* gene overexpression was manifested more strongly. Thus, overexpression of the *VaSTS1* gene significantly increased the quantity of green leaves after UV-C treatment by 2.0 - 2.3 times compared with KA0 *Arabidopsis* plants (Fig. 3*F*). *VaSTS7* gene overexpression also increased the viability of the UV-C irradiated leaves by 1.3 - 1.8 times, but this enhancement was statistically significant for only two of the three Arabidopsis ST7 plants lines that were used in the experiments (for ST7-1 and ST7-3, Fig. 3*F*).

Conclusions

We showed that the tolerance to UV-B and UV-C radiation in transgenic *A. thaliana* plants positively correlated with total stilbene content and *STS* transgene expression. Therefore, *STS* expression led to stilbene accumulation, which had a positive effect on plant tolerance of UV radiation. Thus, we firstly experimentally showed stilbene participation in plant protection from UV radiation. For obtaining *VaSTS1* and *VaSTS7* transgenic plants, we used the same genetic constructs and methods of transformation, but we detected significant differences in *VaSTS1* and *VaSTS7* expression, methylation of the DNA of the *VaSTS1* and *VaSTS7* gene protein coding region, and different stilbene accumulation and stress tolerance. Thus, stilbene accumulation and stress tolerance in *Arabidopsis* plants depends on the nucleotide sequences of the selected *STS* gene, which requires further study.

References

Aleynova, O.A., Grigorchuk, V.P., Dubrovina, A.S., Rybin, V.G., Kiselev, K.V.: Stilbene accumulation in cell cultures of *Vitis amurensis* Rupr. overexpressing *VaSTS1*, *VaSTS2*, and *VaSTS7* genes. - Plant Cell Tissue Organ Cult. **125**: 329-339, 2016.

Buer, C.S., Muday, G.K.: The transparent *testa4* mutation prevents flavonoid synthesis and alters auxin transport and the response of *Arabidopsis* roots to gravity and light. - Plant Cell **16**: 1191-1205, 2004.

Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K., Scheible, W.R.: Genome-wide identification and testing of superios reference genes for trancript normalization in *Arabidopsis*. - Plant Physiol. **139**: 5-17, 2005.

Chong, J., Poutaraud, A., Hugueney, P.: Metabolism and roles of stilbenes in plants. - Plant Sci. **117**: 143-155, 2009.

Duan, D., Halter, D., Baltenweck, R., Tisch, C., Tröster, V., Kortekamp, A., Hugueney, P., Nick, P.: Genetic diversity of stilbene metabolism in *Vitis sylvestris*. - J. exp. Bot. **66**: 3243-3257, 2015.

Dubrovina, A.S., Kiselev, K.V.: Regulation of stilbene biosynthesis in plants. - Planta **346**: 597-623, 2017.

Dubrovina, A.S., Kiselev, K.V., Khristenko, V.S., Aleynova, O.A.: The calcium-dependent protein kinase gene *VaCPK29* is involved in grapevine responses to heat and osmotic stresses. - Plant Growth Regul. **82**:79-89, 2017.

Dubrovina, A.S., Aleynova, O.A., Suprun, A.R., Ogneva, Z.V., Kiselev, K.V.: Transgene suppression in plants by foliar application of *in vitro*-synthesized small interfering RNAs. - Appl. Microbiol. Biotechnol. **104**: 2125-2135, 2020.

Giorcelli, A., Sparvoli, F., Mattivi, F., Tava, A., Balestrazzi, A., Vrhovsek, U., Calligari, P., Bollini, R., Confalonieri, M.: Expression of the stilbene synthase (*StSy*) gene from grapevine in transgenic white poplar results in high accumulation of the antioxidant resveratrol glucosides. - Transgenic Res. **13**: 203-214, 2004.

Hanhineva, K., Kokko, H., Siljanen, H., Rogachev, I., Aharoni, A., Karenlampi, S.O.: Stilbene synthase gene transfer caused alterations in the phenylpropanoid metabolism of transgenic strawberry (*Fragaria* × *ananassa*). - J. exp. Bot. **60**: 2093-2106, 2009.

He, X.C., Xue, F.Y., Zhang, L.L., Guo, H.L., Ma, L.Q., Yang, M.F.: Overexpressing fusion proteins of 4-coumaroyl-CoA ligase (4CL) and stilbene synthase (STS) in tobacco plants leading to resveratrol accumulation and improved stress tolerance. - Plant Biotechnol. Rep. **12**: 295-302, 2018.

Jeandet, P., Delaunois, B., Conreux, A., Donnez, D., Nuzzo, V., Cordelier, S., Clément, C., Courot, E.: Biosynthesis, metabolism, molecular engineering, and biological functions of stilbene phytoalexins in plants. - Biofactors **36**: 331-341, 2010.

Kiselev, K.V.: Perspectives for production and application of resveratrol. - Appl. Microbiol. Biotechnol. **90**: 417-425, 2011.

Kiselev, K.V., Aleynova, O.A.: Influence of overexpression of stilbene synthase *VaSTS7* gene on resveratrol production in transgenic cell cultures of grape *Vitis amurensis* Rupr. - Appl. Biochem. Microbiol. **52**: 56-60, 2016.

Kiselev, K.V., Dubrovina, A.S., Shumakova, O.A., Karetin, Y.A., Manyakhin, A.Y.: Structure and expression profiling of a novel calcium-dependent protein kinase gene, *CDPK3a*, in leaves, stems, grapes, and cell cultures of wild-growing grapevine *Vitis amurensis* Rupr. - Plant Cell Rep. **32**: 431-442, 2013.

Kiselev, K.V., Dubrovina, A.S., Tyunin, A.P.: The methylation status of plant genomic DNA influences PCR efficiency. - J. Plant Physiol. **175**: 59-67, 2015.

Kiselev, K.V., Dubrovina, A.S., Veselova, M.V., Bulgakov, V.P., Fedoreyev, S.A., Zhuravlev, Y.N.: The *rolB* gene-induced overproduction of resveratrol in *Vitis amurensis* transformed cells. - J. Biotechnol. **128**: 681-692, 2007.

Kiselev, K.V., Ogneva, Z.V., Dubrovina, A.S., Nityagovsky, N.N., Suprun, A.R.: Somatic mutations, DNA methylation, and expression of DNA repair genes in *Arabidopsis thaliana* treated with 5-azacytidine. - Biol. Plant. **63**: 398-404, 2019a.

Kiselev, K.V., Ogneva, Z.V., Suprun, A.R., Grigorchuk, V.P., Dubrovina, A.S.: Action of ultraviolet-C radiation and *p*-coumaric acid on stilbene accumulation and expression of stilbene biosynthesis-related genes in the grapevine *Vitis amurensis* Rupr. - Acta Physiol. Plant. **41**: 28, 2019b.

Kobayashi, S., Ding, C.K., Nakamura, Y., Nakajima, I., Matsumoto, R.: Kiwifruits (*Actinidia deliciosa*) transformed with a *Vitis stilbene* synthase gene produce piceid (resveratrol-glucoside). - Plant Cell Rep. **19**: 904-910, 2000.

Laavola, M., Nieminen, R., Leppänen, T., Eckerman, C., Holmbom, B., Moilanen, E.: Pinosylvin and monomethylpinosylvin, constituents of an extract from the knot of *Pinus sylvestris*, reduce inflammatory gene expression and inflammatory responses *in vivo*. - J. Agr. Food Chem. **63**: 3445-3453, 2015.

Langcake, P., Pryce, R.J.: A new class of phytoalexins from grapevines. - Experientia **33**: 151-152, 1977.

Liu, Z., Zhuang, C., Sheng, S., Shao, L., Zhao, W., Zhao, S.: Overexpression of a resveratrol synthase gene (*PcRS*) from *Polygonum cuspidatum* in transgenic *Arabidopsis* causes the accumulation of *trans*-piceid with antifungal activity. - Plant Cell Rep. **30**: 2027-2036, 2011.

Livak, K.J., Schmittgen, T.D.: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. - Methods **25**: 402-408, 2001.

Lo, C., Le, Blanc, J.C.Y., Yu, C.K.Y., Sze, K.H., Ng, D.C.M., Chu, I.K.: Detection, characterization, and quantification of resveratrol glycosides in transgenic *Arabidopsis* over-expressing a sorghum stilbene synthase gene by liquid chromatography/tandem mass spectrometry. - Rapid Commun. Mass. Spectrom. **21**: 4101-4108, 2007.

Luo, Z., Guo, H., Yang, Y., Yang, M., Ma, L., Wang, Y.: Heterologous overexpression of resveratrol synthase (*PcPKS5*) gene enhances antifungal and mite aversion by resveratrol accumulation. - Eur. J. Plant Pathol. **142**: 547-556, 2015.

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

Nawkar, G.M., Maibam, P., Park, J.H., Sahi, V.P., Lee, S.Y., Kang, C.H.: UV-induced cell death in plants. - Int. J. mol. Sci. **14**: 1608-1628, 2013.

Ogneva, Z.V., Dubrovina, A.S., Kiselev, K.V.: Age-associated alterations in DNA methylation and expression of methyltransferase and demethylase genes in *Arabidopsis thaliana*. - Biol. Plant. **60**: 628-634, 2016.

Ogneva, Z.V., Suprun, A.R., Dubrovina, A.S., Kiselev, K.V.: The effect of 5-azacytidine induced DNA demethylation on abiotic stress tolerance in *Arabidopsis thaliana*. - Plant Protect. Sci. **55**: 73-80, 2019.

Rajeevkumar, S., Anunanthini, P., Sathishkumar, R.: Epigenetic silencing in transgenic plants. - Front. Plant Sci. **6**: 693, 2015.

Shankar, S., Nall, D., Tang, S.N., Meeker, D., Passarini, J., Sharma, J., Srivastava, R.K.: Resveratrol inhibits pancreatic cancer stem cell characteristics in human and kras (G12D) transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition. - PLoS ONE **6**: e16530, 2011.

Shumakova, O.A., Manyakhin, A.Y., Kiselev, K.V.: Resveratrol content and expression of phenylalanine ammonia-lyase and stilbene synthase genes in cell cultures of *Vitis amurensis* treated with coumaric acid. - Appl. Biochem. Biotechnol. **165**: 1427-1436, 2011.

Suwalsky, M., Villena, F., Gallardo, M.J.: *In vitro* protective effects of resveratrol against oxidative damage in human erythrocytes. - Biochim. biophys. Acta **1848**: 76-82, 2015.

Tyunin, A.P., Kiselev, K.V.: Alternations in *VaSTS* gene cytosine methylation and *t*-resveratrol production in response to UV-C irradiation in *Vitis amurensis* Rupr. cells. - Plant Cell Tissue Organ Cult. **124**: 33-45, 2016.

Weiskirchen, S., Weiskirchen R.: Resveratrol: how much wine do you have to drink to stay healthy? - Adv. Nutr. **7**: 706-718, 2016.

Yu, C.K.Y., Lam, C.N.W., Springob, K., Schmidt, J., Chu, I.K., Lo, C.: Constitutive accumulation of *cis*-piceid in transgenic *Arabidopsis* overexpressing a sorghum stilbene synthase gene. - Plant Cell Physiol. **47**: 1017-1021, 2006.

Zhang, X.R., Henriques, R., Lin, S.S., Niu, Q.W., Chua, N.H.: *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. - Nature Protocols **1**: 641-646, 2006.