

EFFECT OF DROUGHT, ELEVATED TEMPERATURE, AND THEIR COMBINATION ON PHOTOSYNTHETIC GENES EXPRESSION IN *CHENOPODIUM QUINOA* WILLD. AND *AMARANTHUS RETROFLEXUS* L

¹Shuyskaya E.V., ²Rakhmankulova Z.F., ³Toderich K.N., ⁴Saidova L.T., ⁵Anisina A.A.,
⁶Prokofieva M.Yu.

¹scientific researcher, Timiryazev Institute of Plant Physiology, RAS, Moscow, Russia,
²professor, Timiryazev Institute of Plant Physiology, RAS, Moscow, Russia, ³professor,
Graduate School of Bioresources, Mie University, Japan, ⁴scientific researcher, Timiryazev
Institute of Plant Physiology, RAS, Moscow, Russia, ⁵student, Moscow State University,
Moscow, Russia, ⁶scientific researcher, Timiryazev Institute of Plant Physiology, RAS, Moscow,
Russia

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Annotation. The aridization of the climate implies increasing temperatures and more frequent droughts. For this reason, the study of the effect of these factors separately, and especially of their combined influence, is of great importance. For the study, we have chosen two alternative crops with different photosynthesis type: C_3 species *Chenopodium quinoa* Willd. and C_4 species *Amaranthus retroflexus* L. The expression of genes encoding the main photosynthetic enzymes Rubisco and phosphoenolpyruvate carboxylase PEPC (*rbcL*, *rbcS*, *Ppc1* and *Ppc2*), major components of photosystems (PS) I and II (*psaA*, *psaB* and *psbA*), the main (PRG5) and the second (*ndhD(B)*) pathways of cyclic electron transport around photosystem I (CET PS I) and chlorophyll *a/b*-binding protein LHCB/CAB PS II (LHCB) under drought, elevated temperature (eT), and their combination was studied. Our results showed that drought negatively affected the expression of gene, which codes photosynthetic enzymes: Rubisco in both species, and the C_4 enzyme PEPC in C_4 species *A. retroflexus*. In *A. retroflexus*, decreased expression of the *ndhB* gene of the second CET PS I pathway associated with the C_4 cycle was also observed. Under elevated temperature *rbcS* expression was increased in *C. quinoa*, which is probably related to the regulatory function of Rubisco small subunit. Moreover, upregulation of LHCB (chlorophyll *a/b*-binding protein) and PRG5 (the main CET PS I pathway) expression may be a protective response to maintain the activity of linear and cyclic electron transport. Elevated temperature had a more negative effect on the expression of genes encoding PS I and PS II components in *A. retroflexus* than drought. At the same time, eT did not decrease the expression of C_4 -enzyme PEPC genes and stimulated the expression of the gene of the second CET PS I pathway associated with C_4 cycle (*ndhB*) in *A. retroflexus*. The combined effect of the two factors created a synergistic effect on the expression of *Ppc1,2* and *psaA,B* in *C. quinoa*, and *rbcL* in *A. retroflexus*. In general, in *C. quinoa*, the genes encoding PS I components were most sensitive to the combined effect of the two factors, which may lead to a decrease in PS I efficiency. In *A. retroflexus*, the combined effect of the two factors mitigated the negative effect of drought on PS I components genes and upregulated the expression of two CET PS I pathways genes, presumably to maintain the required level of CET PS I activity.

Key words: *Chenopodium quinoa*, *Amaranthus retroflexus*, drought, elevated temperatures, photosynthetic genes

INTRODUCTION

Climate aridization implies increasing temperatures and more frequent droughts. It is suggested that the combined action of two stressors causes a stronger effect than the action of individual factors. Moreover, plant responses to combined stresses can be unique and/or antagonistic to single stresses [1]. Plant adaptation to combined stresses requires a specific combined response, including physiological, biochemical, and molecular reactions [2]. Both of these factors negatively affect plant photosynthesis, both at the level of light and dark reactions and the expression of photosynthetic genes. If drought acts more strongly on enzymes, then photosystems and electron transport are considered to be particularly sensitive to temperature increases [3].

For optimal photosynthesis under any conditions, it is necessary to maintain an energy balance, which includes balancing the ratio of production and consumption of ATP to NADPH in cells. Under stress, this balance is disrupted, which leads to the formation of active oxygen species in both photosystems. Plants have various photoprotective mechanisms that maintain the energy balance of chloroplasts, including cyclic electron transport around PS I (CET PS I) [4, 5]. Two cyclic electron transport pathways around PS I are known: the PGR5/PGRL1 protein-dependent pathway and the NADH dehydrogenase-like (NDH) complex-dependent pathway. PGR5/PGRL1 is considered the major CET pathway in C₃ plants, and the NDH-complex-dependent pathway in C₄ species, but both pathways contribute to CET and may partially compensate for each other's activity [4, 6]. In addition, it is considered that two additional ATP molecules are produced during CET PS I, which are necessary for the activity of the C₄ cycle in C₄ plant species.

We aimed to study the effect of drought, elevated temperatures, and their combination on the expression of photosynthetic genes of two perspective crops of the Amaranthaceae family with different photosynthesis types: C₃ species *Chenopodium quinoa* Willd. and C₄ species *Amaranthus retroflexus* L., which are characterized by stress resistance and high nutritional value of seeds.

MATERIAL AND METHODS

Seedlings of *Chenopodium quinoa* Willd. and *Amaranthus retroflexus* L. were grown for 30 days in plastic pots filled with perlite. The seedlings were irrigated with a 50% Hoagland nutrient solution. The light regime consisted of 8 h dark/16 h light (200 mmol/(m² s) PAR), and the temperature was maintained at 25°C. After a 30-day period, some plants were subjected a 4-day treatment with elevated temperature (35°C), some plants were subjected a 4-day treatment with a 12.5% (m/v) solution of PEG 6000, and some plants were subjected a 4-day treatment with both elevated temperature and PEG 6000 solution.

Total RNA was extracted from the leaf samples (0.50 g FW) using phenol–chloroform extraction with precipitation using LiCl as described previously [68]. Reverse transcription was performed according to the standard Evrogen protocol (Evrogen, Moscow, Russia). PCR primers were designed using Pick Primers NCBI (National Center for Biotechnology Information, Bethesda, MD, USA) with Primer Pair Specificity Checking Parameters and SnapGene Viewer (4.2.11) on nucleotide sequences available in the NCBI database. The transcript levels were assessed with real-time quantitative PCR (qRT-PCR) using a LightCycler 96 amplifier (Roche, Basel, Switzerland) with SybrGreen I dye (Evrogen, Moscow, Russia). qRT-PCR data were analyzed using LightCycler 96 Software Version 1.1. Relative quantification was performed to compare the levels of the target gene and the reference gene, and the result was expressed as a ratio. UBQ10 and b-Tubulin were used as reference genes. Transcript levels were calculated relative to control plants.

RESULTS

Drought downregulated *rbcL* and *RbcS* genes of the main photosynthetic enzyme Rubisco in both species and the *Ppc1* gene encoding the C₄ enzyme PEPC in C₄ species *A. retroflexus* (Figure). In addition, drought downregulated the *ndhB* gene encoding the component of the second pathway CET PS I in *A. retroflexus*.

Elevated temperature also decreased Rubisco gene expression (*rbcL*, *RbcS*) in *A. retroflexus* but not in *C. quinoa*. Moreover, in *C. quinoa*, *RbcS* expression was upregulated by eT. Elevated temperature had a greater effect on genes encoding PS I, PS II, and CET PS I components in both species. So, in *C. quinoa*, upregulation of *PGR5* and *LHCB* expression was observed. Whereas in *A. retroflexus*, eT negatively affected the expression of *psaA*, *psaB*, and *psbA* genes encoding the PS I and PS II components, but increased the *ndhB* expression (component of second CET PS I pathway).

Combined effect of drought and elevated temperatures led to decreased expression of *rbcL* (as drought alone) and no changes in *RbcS* expression (as a result of the opposite effects of drought and eT separately) in *C. quinoa*. The synergistic effect of the combined action of two factors was manifested in the increased expression of both PEPC isoforms (*Ppc1* and *Ppc2*), which was not observed when these factors were applied separately in *C. quinoa*.

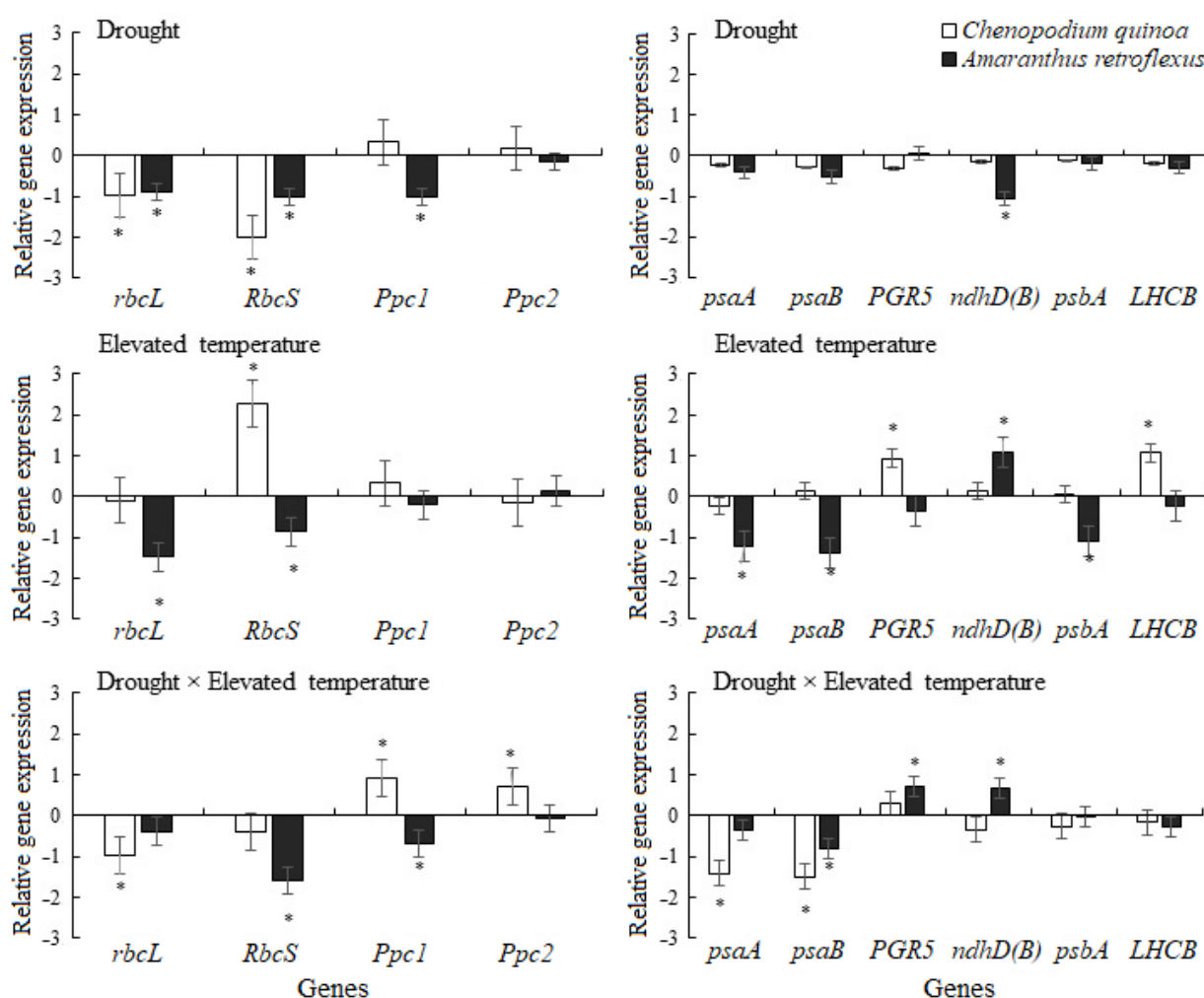


Figure. The expression of genes encoding the main photosynthetic enzymes Rubisco and PEPC (*rbcL*, *rbcS*, *Ppc1* and *Ppc2*), major components of PS I and PS II (*psaA*, *psaB* and *psbA*),

the main (*PRG5*) and the second (*ndhD(B)*) pathways of CET PS I and chlorophyll *a/b*-binding protein LHCB/CAB PS II (*LHCB*) under drought, elevated temperature, and their combination.

In *A. retroflexus*, there was found the synergistic effect of the combined action of two factors on *rbcL* expression (no change, whereas a decrease in expression was observed with the separate action of these factors) and the accumulative effect on *RbcS* expression (increased expression compared to the separate actions of the factors). *Ppc1* expression was also decreased as in the case of drought action alone.

Combined effect of drought and elevated temperatures negatively affected the expression of *psaA* and *psaB* genes encoding PS I and PS II components as opposed to the separate action of these factors in *C. quinoa*. However, no upregulation of *PGR5* and *LHCB* expression was observed as under eT alone in *C. quinoa*. In *A. retroflexus*, the combined action of two factors decreased negative effects of eT on *psaA*, *psaB* and *psbA* expression. At the same time, the upregulation of expression of both *PRG5* and *ndhB* genes encoding components of two ways of CET PS I was observed in *A. retroflexus*.

CONCLUSIONS

The results of our research on the effect of drought, elevated temperature, and their combined effect showed that drought downregulated photosynthetic enzyme genes but not photosystems I and II components in both species, *C. quinoa* and *A. retroflexus*. In addition, drought was found to negatively affect the expression of C₄ cycle-related genes in *A. retroflexus*.

Elevated temperature did not have such a negative effect on photosynthetic gene expression in the C₃ species *C. quinoa* as it did in the C₄ species *A. retroflexus*. In *C. quinoa*, *rbcS* expression was increased, which is probably related to the regulatory function of Rubisco small subunit. Moreover, the upregulation of the *LHCB* gene encoding chlorophyll *a/b*-binding protein LHCB/CAB PS II and the *PRG5* gene encoding the component of the main CET PS I pathway may be a protective response to maintain the activity of linear and cyclic electron transport in *C. quinoa*. Elevated temperature had a more negative effect on the expression of genes encoding PS I and PS II components in *A. retroflexus* than drought, but not on the expression of C₄ cycle-related genes.

The combined effect of the two factors generated some synergistic effects in both species. In general, in *C. quinoa*, the genes encoding PS I components were most sensitive to the combined effect of the two factors, which may lead to a decrease in PS I efficiency. In *A. retroflexus*, the combined effect of the two factors mitigated the negative effect of drought on PS I genes and upregulated the expression of *PRG5* and *ndhB* genes of the two CET PS I pathways, probably to maintain the CET PS I activity required for the functioning of the C₄ cycle.

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