

PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF THE ANTIOXIDANT ACTIVITY AND CYTOTOXICITY POTENTIAL OF HYDROETHANOLIC EXTRACT OF BULGARIAN *SIDERITIS SCARDICA*

Anelia Dobrikova¹, Zlatina Gospodinova^{1,2}, Martin Stefanov¹, Emilia Apostolova¹, Natalia Krasteva¹

¹*Institute of Biophysics and Biomedical Engineering
Bulgarian Academy of Sciences
"Acad. Georgi Bonchev" Str., bl. 21, Sofia 1113, Bulgaria*

²*Institute of Plant Physiology and Genetics
Bulgarian Academy of Sciences
"Acad. Georgi Bonchev" Str., bl. 21, Sofia 1113, Bulgaria
E-mail: nataly@bio21.bas.bg*

Received 09 April 2022

Accepted 27 May 2022

ABSTRACT

Sideritis scardica (S. scardica) is a mountain plant, endemic to the central part of the Balkan Peninsula, including the southern regions of Bulgaria. It is widely used in traditional medicine to treat some respiratory, gastrointestinal tract, or neurodegenerative diseases, but research data for its cytotoxic and anticancer properties are limited. In the present study, a hydroethanolic extract of S. scardica from region Trigrad, Bulgaria was prepared and its total polyphenolic and flavonoid content was determined. Further, its antioxidant, cytotoxic, and anticancer activities toward a mouse adenocarcinoma cell line, Colon 26 was evaluated in vitro. Higher antioxidant activity and higher total phenolic and flavonoid content were detected in the S. scardica hydroethanolic extract from region Trigrad as compared to the published data for other ecotypes of this species. Also, the extract of S. scardica with a concentration higher than 200 µg mL⁻¹ was found to inhibit the viability and growth of colon cancer cells but not those of the normal kidney MDCK cells pointing to a cell-specific and concentration-dependent cytotoxic effect. Thus, the current study demonstrates a high oxidation potential of S. scardica hydroethanolic extract from the region Trigrad and its cytotoxic effects on colon cancer cells when the extract was applied with concentrations of 200 µg mL⁻¹ and higher.

Keywords: total phenolic and flavonoid content, anticancer activity, MDCK cells, Colon 26 cancer cells.

Article

The Role of Alternative Electron Pathways for Effectiveness of Photosynthetic Performance of *Arabidopsis thaliana*, Wt and *Lut2*, under Low Temperature and High Light Intensity

Antoaneta V. Popova ^{1,*} , Martin Stefanov ¹, Alexander G. Ivanov ^{1,2}  and Maya Velitchkova ¹ 

¹ Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

² Department of Biology, University of Western Ontario, 1151 Richmond Str. N., London, ON N6A 5B7, Canada

* Correspondence: popova@bio21.bas.bg

Abstract: A recent investigation has suggested that the enhanced capacity for PSI-dependent cyclic electron flow (CEF) and PSI-dependent energy quenching that is related to chloroplast structural changes may explain the lower susceptibility of *lut2* to combined stresses—a low temperature and a high light intensity. The possible involvement of alternative electron transport pathways, proton gradient regulator 5 (PGR5)-dependent CEF and plastid terminal oxidase (PTOX)-mediated electron transfer to oxygen in the response of *Arabidopsis* plants—wild type (wt) and *lut2*—to treatment with these two stressors was assessed by using specific electron transport inhibitors. Re-reduction kinetics of P_{700}^+ indicated that the capacity for CEF was higher in *lut2* when this was compared to wt. Exposure of wt plants to the stress conditions caused increased CEF and was accompanied by a substantial raise in PGR5 and PTOX quantities. In contrast, both PGR5 and PTOX levels decreased under the same stress conditions in *lut2*, and inhibiting PGR5-dependent pathway by AntA did not exhibit any significant effects on CEF during the stress treatment and recovery period. Electron microscopy observations demonstrated that under control conditions the degree of grana stacking was much lower in *lut2*, and it almost disappeared under the combined stresses, compared to wt. The role of differential responses of alternative electron transport pathways in the acclimation to the stress conditions that are studied is discussed.

Keywords: alternative electron flows; combined abiotic stress; carotenoid mutant; cyclic electron transport; PGR5; photoprotection; PTOX



Citation: Popova, A.V.; Stefanov, M.; Ivanov, A.G.; Velitchkova, M. The Role of Alternative Electron Pathways for Effectiveness of Photosynthetic Performance of *Arabidopsis thaliana*, Wt and *Lut2*, under Low Temperature and High Light Intensity. *Plants* **2022**, *11*, 2318. <https://doi.org/10.3390/plants11172318>

Academic Editor: Georgia Ouzounidou

ASSESSMENT OF THE IMPACT OF SALINITY ON
PHOTOSYNTHETIC APPARATUS OF *PAULOWNIA*
ELONGATA x *KAWAKAMI*

Martin Stefanov, Ekaterina Yotsova, Katya Ivanova*,
Yuliana Markovska*, Emilia Apostolova

(Submitted by Academician K. Koumanov on May 5, 2016)

Abstract

The impact of the salinity on the functional activity of photosynthetic apparatus and pigment composition of *Paulownia elongata* x *kawakami* grown in soils with different salinity was investigated using PAM chlorophyll fluorescence and steady-state P₇₀₀ photo-oxidation by far-red light. Increase of the soil salinity stimulated the PSII activity while PSI activity was not influenced. Data also revealed increase of the chlorophylls and carotenoids in the plant grown in saline soil. The high salt tolerance of the *Paulownia elongata* x *kawakami* is discussed.

Key words: *Paulownia*, salinity, chlorophyll fluorescence, P₇₀₀, pigment composition

This work was supported by the Bulgarian Academy of Sciences and the Program for career development of young scientists, BAS.

Chapter title:

Responses of Photosynthetic Apparatus to Salt Stress: Structure, Function and Protection

M. Stefanov¹, A.K. Biswal^{2,3,4}, M. Misra⁵, A. N. Misra^{5,6}
E.L. Apostolova^{1*}

¹Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences,
Acad.G. Bonchev Str. 21, Sofia 1113, Bulgaria

²Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, TN 37831,
USA

³Complex Carbohydrate Research Center, University of Georgia, Athens, GA

⁴Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA
e-mail: biswalajaya@ccrc.uga.edu

⁵Khallikote University, Berhampur-760001, Odisha State, India.

⁶Center for Life Sciences, Central University of Jharkhand, Ratu-Lohardaga Road, Brambe-
835205, Ranchi, Jharkhand State, India.

e-mail: misra.amarendra@gmail.com, misran@yahoo.co.uk

*Corresponding author:

Emilia L. Apostolova
Institute of Biophysics and Biomedical Engineering,
Bulgarian Academy of Sciences,
Acad. G. Bonchev Str. 21
1113 Sofia, Bulgaria
Tel: +359-2 979 26 21
Fax: +359-2 971 24 93
e-mail address: emya@bio21.bas.bg,
emilia.apostolova@gmail.com

Abstract

Salt stress is one of the abiotic stresses that limits plant growth and yield of plants world wide, and have a strong influence on the photosynthesis. It is known that high salt concentrations alter on the protein composition, the amount of pigments, the total lipid and the fatty acid content of the photosynthetic apparatus, which is accompanied by changes in the structure and function of chloroplast. Electron microscopic studies have shown that increasing the amount of salt leads to the destruction of chloroplast envelope, a damage to stromal thylakoids and a disintegration of the granal thylakoid systems. All these changes decrease the efficiency of photosynthesis, as the effects depend on the plant species as well as the dose and the duration of stress. The studies of plants with different sensitivity to salt stress as well as their defence mechanisms is an important step in better understanding how plants can acclimate to the adverse environments like salt and other stresses. In this chapter, we review the effects of salt stress on the structure and the function of the photosynthetic apparatus as well as the defence mechanisms in plants focusing mostly on the antioxidant defence system and the osmolites.

I. Introduction

II. Organization of the photosynthetic apparatus

III. Effects of the salt stress on the plants

III.1. Plant growth, development and yields

III.2. Photosynthetic pigments

III.3. Lipid composition

III.4. Chloroplasts and thylakoid membranes

III.4.1. Structure

III.4.2. Function

IV. Defense mechanism in the plants again the salt stress

IV.1. Antioxidative defense system

IV.2. Xanthophyll cycle

IV.2. Accumulation of the osmolytes

V. Conclusions

References

ECOLOGIA BALKANICA

2020, Special Edition 3

pp. 147-154

Effects of Salt Stress on the Photosynthesis of Maize and Sorghum

*Martin A. Stefanov, Georgi D. Rashkov, Ekaterina K. Yotsova,
Preslava B. Borisova, Anelia G. Dobrikova, Emilia L. Apostolova**

Bulgarian Academy of Sciences, Institute of Biophysics and Biomedical Engineering,
1113 Sofia, BULGARIA


*Corresponding author: emya@bio21.bas.bg

Abstract. In this study, the effects of salt stress on the photosynthetic processes in sorghum (*Sorghum bicolor* L. *Albanus concept*) and maize (*Zea mays* L. *Mayflower*) were compared. The plants were grown in half-strength Hoagland solutions containing different NaCl concentrations (0, 50, 150 and 250 mM NaCl) for 6 days. Pulse Amplitude Modulated chlorophyll fluorescence, photooxidation of P₇₀₀ and pigment analysis were used for characterization of the salinity effects on the studied plants. The treatment of plants with the high concentrations of NaCl led to an inhibition of the chlorophyll fluorescence parameters like the photochemical quenching, the rate of photosynthesis and the linear electron transport in sorghum and maize. All these changes corresponded to decrease in the pigment content and changes in chlorophyll *a/b* ratio. The analysis of the P₇₀₀ photooxidation revealed that the photosystem I photochemistry was inhibited at the highest NaCl concentration in both studied plants. Data also revealed that sorghum is more sensitive to salt stress than maize. The reasons for different effects of salt stress on the maize and sorghum are described.

Key words: chlorophyll fluorescence, pigment content, salt stress, sorghum, maize.

Article

Different Sensitivity Levels of the Photosynthetic Apparatus in *Zea mays* L. and *Sorghum bicolor* L. under Salt Stress

Martin A. Stefanov, Georgi D. Rashkov, Ekaterina K. Yotsova, Preslava B. Borisova, Anelia G. Dobrikova  and Emilia L. Apostolova *

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Street, Block 21, 1113 Sofia, Bulgaria; martin_12.1989@abv.bg (M.A.S.); megajorko@abv.bg (G.D.R.); ekaterina_yotsova@abv.bg (E.K.Y.); preslavab12345@gmail.com (P.B.B.); aneli@bio21.bas.bg (A.G.D.)

* Correspondence: emya@bio21.bas.bg or emilia.apostolova@gmail.com

Abstract: The impacts of different NaCl concentrations (0–250 mM) on the photosynthesis of new hybrid lines of maize (*Zea mays* L. Kerala) and sorghum (*Sorghum bicolor* L. Shamal) were investigated. Salt-induced changes in the functions of photosynthetic apparatus were assessed using chlorophyll *a* fluorescence (PAM and OJIP test) and P₇₀₀ photooxidation. Greater differences between the studied species in response to salinization were observed at 150 mM and 200 mM NaCl. The data revealed the stronger influence of maize in comparison to sorghum on the amount of closed PSII centers (1-qp) and their efficiency (Φ_{exc}), as well as on the effective quantum yield of the photochemical energy conversion of PSII (Φ_{PSII}). Changes in the effective antenna size of PSII (ABS/RC), the electron flux per active reaction center (REo/RC) and the electron transport flux further Q_A (ETo/RC) were also registered. These changes in primary PSII photochemistry influenced the electron transport rate (ETR) and photosynthetic rate (parameter R_{Fd}), with the impacts being stronger in maize than sorghum. Moreover, the lowering of the electron transport rate from Q_A to the PSI end electron acceptors (REo/RC) and the probability of their reduction (φ_{Ro}) altered the PSI photochemical activity, which influenced photooxidation of P₇₀₀ and its decay kinetics. The pigment content and stress markers of oxidative damage were also determined. The data revealed a better salt tolerance of sorghum than maize, associated with the structural alterations in the photosynthetic membranes and the stimulation of the cyclic electron flow around PSI at higher NaCl concentrations. The relationships between the decreased pigment content, increased levels of stress markers and different inhibition levels of the function of both photosystems are discussed.



Citation: Stefanov, M.A.; Rashkov, G.D.; Yotsova, E.K.; Borisova, P.B.; Dobrikova, A.G.; Apostolova, E.L. Different Sensitivity Levels of the Photosynthetic Apparatus in *Zea mays* L. and *Sorghum bicolor* L. under Salt Stress. *Plants* **2021**, *10*, 1469. <https://doi.org/10.3390/plants10071469>

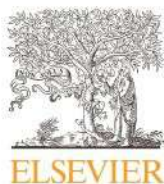
Academic Editor: Fermín Morales

Received: 12 June 2021

Accepted: 13 July 2021

Published: 17 July 2021

Keywords: NaCl treatment; OJIP test; PAM chlorophyll fluorescence; photosynthesis; pigment composition; stress markers



Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb



Role of flavonoids and proline in the protection of photosynthetic apparatus in *Paulownia* under salt stress



Martin Stefanov^a, Ekaterina Yotsova^a, Emilia Gesheva^b, Velmira Dimitrova^{c,d},
Yuliana Markovska^c, Snezhanka Doncheva^b, Emilia L. Apostolova^{a,*}

^a Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. 21, 1113 Sofia, Bulgaria

^b Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria

^c Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

^d Biotree LTD, 7 Bansko shose Str, Sofia, Bulgaria

ARTICLE INFO

Article History:

Received 17 June 2020

Revised 17 December 2020

Accepted 14 February 2021

Available online 18 March 2021

Edited by S Barnard

Keywords:

Antioxidant activity

Flavonoids

Paulownia

Photosynthetic apparatus

Proline

Salt stress

ABSTRACT

The effects of different NaCl concentrations (0–150 mM) for different periods of time (10 days and 25 days) on *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *elongata* were analyzed. The decrease of the pigment composition, an inhibition of the photochemistry of both photosystems as well as an increase of the antioxidant activity (FRAP assay), the antiradical activity (DPPH assay), the total flavonoids and the proline levels were affected only at short salt treatment (10 days) with high NaCl concentration (150 mM). These changes correspond with some decrease of the maximum quantum yields of photosystem II (Fv/Fm), the effective quantum yield of photochemical energy conversion (Φ_{PSII}) and the photochemical quenching (q_P) as a result of a restriction of electron flow from Q_A to Q_B as well as an influence on the oxidation reduction properties of P_{700} . The role of the carotenoids and levels of flavonoids and proline during the first days of salt stress for the prevention of the functions of the photosynthetic apparatus and adaptation of plants to different high salt contents are discussed.

© 2021 SAAB. Published by Elsevier B.V. All rights reserved.



Article

Assessment of the Photosynthetic Apparatus Functions by Chlorophyll Fluorescence and P_{700} Absorbance in C3 and C4 Plants under Physiological Conditions and under Salt Stress

Martin A. Stefanov, Georgi D. Rashkov and Emilia L. Apostolova *

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria; martin_12.1989@abv.bg (M.A.S.); megajorko@abv.bg (G.D.R.)

* Correspondence: emya@bio21.bas.bg or emilia.apostolova@gmail.com

Abstract: Functions of the photosynthetic apparatus of C3 (*Pisum sativum* L.) and C4 (*Zea mays* L.) plants under physiological conditions and after treatment with different NaCl concentrations (0–200 mM) were investigated using chlorophyll a fluorescence (pulse-amplitude-modulated (PAM) and JIP test) and P_{700} photooxidation measurement. Data revealed lower density of the photosynthetic structures (RC/CSo), larger relative size of the plastoquinone (PQ) pool (N) and higher electron transport capacity and photosynthetic rate (parameter R_{Fd}) in C4 than in C3 plants. Furthermore, the differences were observed between the two studied species in the parameters characterizing the possibility of reduction in the photosystem (PSI) end acceptors (RE_o/RC , RE_o/CSo and δRo). Data revealed that NaCl treatment caused a decrease in the density of the photosynthetic structures and relative size of the PQ pool as well as decrease in the electron transport to the PSI end electron acceptors and the probability of their reduction as well as an increase in the thermal dissipation. The effects were stronger in pea than in maize. The enhanced energy losses after high salt treatment in maize were mainly from the increase in the regulated energy losses (Φ_{NPQ}), while in pea from the increase in non-regulated energy losses (Φ_{NO}). The reduction in the electron transport from Q_A to the PSI end electron acceptors influenced PSI activity. Analysis of the P_{700} photooxidation and its decay kinetics revealed an influence of two PSI populations in pea after treatment with 150 mM and 200 mM NaCl, while in maize the negligible changes were registered only at 200 mM NaCl. The experimental results clearly show less salt tolerance of pea than maize.

Keywords: NaCl treatment; JIP test; PAM chlorophyll fluorescence; pea; photooxidation of P_{700} ; maize



Citation: Stefanov, M.A.; Rashkov, G.D.; Apostolova, E.L. Assessment of the Photosynthetic Apparatus Functions by Chlorophyll Fluorescence and P_{700} Absorbance in C3 and C4 Plants under Physiological Conditions and under Salt Stress. *Int. J. Mol. Sci.* **2022**, *23*, 3768. <https://doi.org/10.3390/ijms23073768>

Academic Editors: Giampiero Cai, Luigi Parrotta and Lavinia Mareri



Article

Impact of Salinity on the Energy Transfer between Pigment–Protein Complexes in Photosynthetic Apparatus, Functions of the Oxygen-Evolving Complex and Photochemical Activities of Photosystem II and Photosystem I in Two *Paulownia* Lines

Martin A. Stefanov, Georgi D. Rashkov, Ekaterina K. Yotsova, Anelia G. Dobrikova  and Emilia L. Apostolova * 

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

* Correspondence: emya@bio21.bas.bg or emilia.apostolova@gmail.com

Abstract: The present study shows the effect of salinity on the functions of thylakoid membranes from two hybrid lines of *Paulownia*: *Paulownia tomentosa* × *fortunei* and *Paulownia elongata* × *elongata*, grown in a Hoagland solution with two NaCl concentrations (100 and 150 mM) and different exposure times (10 and 25 days). We observed inhibition of the photochemical activities of photosystem I ($\text{DCPIH}_2 \rightarrow \text{MV}$) and photosystem II ($\text{H}_2\text{O} \rightarrow \text{BQ}$) only after the short treatment (10 days) with the higher NaCl concentration. Data also revealed alterations in the energy transfer between pigment–protein complexes (fluorescence emission ratios F_{735}/F_{685} and F_{695}/F_{685}), the kinetic parameters of the oxygen-evolving reactions (initial S_0 – S_1 state distribution, misses (α), double hits (β) and blocked centers (S_B)). Moreover, the experimental results showed that after prolonged treatment with NaCl *Paulownia tomentosa* × *fortunei* adapted to the higher concentration of NaCl (150 mM), while this concentration is lethal for *Paulownia elongata* × *elongata*. This study demonstrated the relationship between the salt-induced inhibition of the photochemistry of both photosystems and the salt-induced changes in the energy transfer between the pigment–protein complexes and the alterations in the Mn cluster of the oxygen-evolving complex under salt stress.

Keywords: electron transport; low-temperature chlorophyll fluorescence; thylakoid membranes; NaCl treatment



Citation: Stefanov, M.A.; Rashkov, G.D.; Yotsova, E.K.; Dobrikova, A.G.; Apostolova, E.L. Impact of Salinity on the Energy Transfer between Pigment–Protein Complexes in Photosynthetic Apparatus, Functions of the Oxygen-Evolving Complex and Photochemical Activities of Photosystem II and Photosystem I in

Article

Sensitivity of the Photosynthetic Apparatus in Maize and Sorghum under Different Drought Levels

Martin Stefanov, Georgi Rashkov, Preslava Borisova and Emilia Apostolova *

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria; martin_12.1989@abv.bg (M.S.); megajorko@abv.bg (G.R.); preslavab12345@gmail.com (P.B.)

* Correspondence: emya@bio21.bas.bg or emilia.apostolova@gmail.com

Abstract: Drought is one of the main environmental stress factors affecting plant growth and yield. The impact of different PEG concentrations on the photosynthetic performance of maize (*Zea mays* L. Mayflower) and sorghum (*Sorghum bicolor* L. Foehn) was investigated. The activity of the photosynthetic apparatus was assessed using chlorophyll fluorescence (PAM and JIP test) and photooxidation of P_{700} . The data revealed that water deficiency decreased the photochemical quenching (qP), the ratio of photochemical to nonphotochemical processes (F_v/F_o), the effective quantum yield of the photochemical energy conversion in PSII (Φ_{PSII}), the rate of the electron transport (ETR), and the performance indexes PI_{total} and PI_{ABS} , as the impact was stronger in sorghum than in maize and depended on drought level. The PSI photochemistry (P_{700} photooxidation) in sorghum was inhibited after the application of all studied drought levels, while in maize, it was registered only after treatment with higher PEG concentrations (30% and 40%). Enhanced regulated energy losses (Φ_{NPQ}) and activation of the state transition under drought were also observed in maize, while in sorghum, an increase mainly in nonregulated energy losses (Φ_{NO}). A decrease in pigment content and relative water content and an increase in membrane damage were also registered after PEG treatment. The experimental results showed better drought tolerance of maize than sorghum. This study provides new information about the role of regulated energy losses and state transition for the protection of the photosynthetic apparatus under drought and might be a practical approach to the determination of the drought tolerance of plants.

Citation: Stefanov, M.; Rashkov, G.; Borisova, P.; Apostolova E.



Sensitivity of the Photosynthetic Apparatus in Maize and Sorghum under Different Drought Levels.

Plants **2023**, *12*, 1863. <https://doi.org/10.3390/plants12091863>

Keywords: chlorophyll fluorescence; PEG treatment; P_{700} photooxidation; pigment composition; membrane injury; maize; sorghum; relative water content

Article

Protective Effects of Sodium Nitroprusside on Photosynthetic Performance of *Sorghum bicolor* L. under Salt Stress

Martin A. Stefanov, Georgi D. Rashkov, Ekaterina K. Yotsova, Preslava B. Borisova, Anelia G. Dobrikova  and Emilia L. Apostolova * 

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

* Correspondence: emya@bio21.bas.bg

Abstract: In this study, the impacts of the foliar application of different sodium nitroprusside (SNP, as a donor of nitric oxide) concentrations (0–300 μM) on two sorghum varieties (*Sorghum bicolor* L. Albanus and *Sorghum bicolor* L. Shamal) under salt stress (150 mM NaCl) were investigated. The data revealed that salinity leads to an increase in oxidative stress markers and damage of the membrane integrity, accompanied by a decrease in the chlorophyll content, the open photosystem II (PSII) centers, and the performance indexes (PI_{ABS} and PI_{total}), as well as having an influence on the electron flux reducing photosystem I (PSI) end acceptors (REo/RC). Spraying with SNP alleviated the NaCl toxicity on the photosynthetic functions; the protection was concentration-dependent, and greater in Shamal than in Albanus, i.e., variety specific. Furthermore, the experimental results revealed that the degree of SNP protection under salt stress also depends on the endogenous nitric oxide (NO) amount in leaves, the number of active reaction centers per PSII antenna chlorophylls, the enhanced electron flux reducing end acceptors at the acceptor side of PSI, as well as the stimulation of the cyclic electron transport around PSI. The results showed better protection in both varieties of sorghum for SNP concentrations up to 150 μM , which corresponds to about a 50% increase in the endogenous NO leaf content in comparison to the control plants. Our study provides valuable insight into the molecular mechanisms underlying SNP-induced salt tolerance in sorghum varieties and might be a practical approach to correcting salt intolerance.



Citation: Stefanov, M.A.; Rashkov, G.D.; Yotsova, E.K.; Borisova, P.B.; Dobrikova, A.G.; Apostolova, E.L. Protective Effects of Sodium Nitroprusside on Photosynthetic Performance of *Sorghum bicolor* L. under Salt Stress. *Plants* **2023**, *12*, 832.

Keywords: NaCl treatment; nitric oxide; photosynthesis; JIP test; chlorophyll fluorescence; membrane damage; P700 photooxidation

Ekaterina K. Yotsova, Martin A. Stefanov, Anelia G. Dobrikova and Emilia L. Apostolova*

Different sensitivities of photosystem II in green algae and cyanobacteria to phenylurea and phenol-type herbicides: effect on electron donor side

DOI 10.1515/znc-2016-0089

Received May 10, 2016; revised January 25, 2017; accepted February 5, 2017

Abstract: The effects of short-term treatment with phenylurea (DCMU, isotroturon) and phenol-type (ioxynil) herbicides on the green alga *Chlorella kessleri* and the cyanobacterium *Synechocystis salina* with different organizations of photosystem II (PSII) were investigated using pulse amplitude modulated (PAM) chlorophyll fluorescence and photosynthetic oxygen evolution measured by polarographic oxygen electrodes (Clark-type and Joliot-type). The photosynthetic oxygen evolution showed stronger inhibition than the PSII photochemistry. The effects of the studied herbicides on both algal and cyanobacterial cells decreased in the following order: DCMU > isotroturon > ioxynil. Furthermore, we observed that the number of blocked PSII centers increased significantly after DCMU treatment (204–250 times) and slightly after ioxynil treatment (19–35 times) in comparison with the control cells. This study suggests that the herbicides affect not only the acceptor side but also the donor side of PSII by modifications of the Mn cluster of the oxygen-evolving complex. We propose that one of the reasons for the different PSII inhibitions caused by herbicides is their influence, in different extents, on the kinetic parameters of the oxygen-evolving reactions (the initial $S_0 - S_1$ state distribution, the number of blocked centers S_B , the turnover time of S_1 states, misses and double hits). The relationship between the herbicide-induced inhibition and the changes in the kinetic parameters is discussed.

*Corresponding author: Emilia L. Apostolova, Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria, E-mail: emya@bio21.bas.bg, Tel: + 359-2979-2621, Fax: + 359-2971-2493

Ekaterina K. Yotsova, Martin A. Stefanov and Anelia G. Dobrikova: Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria

Keywords: *Chlorella kessleri*; herbicides; PAM chlorophyll fluorescence; photosynthetic oxygen evolution; *Synechocystis salina*.

1 Introduction

The application of herbicides in agricultural practice leads to an increase of pollution levels in soil and water, causing environmental problems. Their high toxicity, even at low concentrations, is dangerous for all living organisms. A number of herbicides with an agricultural importance are reported to act on photosystem II (PSII). They can be differentiated into two groups according to their chemical specification and inhibitory patterns: urea/triazine and phenol-type herbicides [1]. PSII is a major multisubunit chlorophyll-protein complex embedded in the thylakoid membrane, which drives electron transfer from water to plastoquinone (PQ) to produce molecular oxygen and protons using energy derived from light [2–4]. The herbicides, affecting the functions of PSII, inhibit electron transfer from Q_A to Q_B due to the competition of herbicides with PQ binding to the exchangeable Q_B site in the PSII complex [5, 6]. An accumulation of reduced Q_A , as a result from blocking of the electron transfer from Q_A to Q_B , leads to an increase in the proportion of Q_B nonreducing centers and an influence of the PSII function. It is known that the phenylurea-type and phenol-type herbicides interact with different amino acid residues on the D1 protein of PSII [7].

Moreover, Ajlani et al. [8] have suggested that the phenol-type herbicide ioxynil inhibits both the acceptor and the donor side of PSII. In addition, studies with mutants presupposed that asparagine 266, valine 249, phenylalanine 255 and alanine 251 participate in the binding niche of ioxynil [8]. It has also been shown that the mutant of *Synechocystis* 6714, in which asparagine at position 266 of the D1 protein is replaced with tyrosine, is resistant to ioxynil but not to the phenylurea herbicide DCMU [8]. Later studies on the double mutant of *Synechocystis* 6714 with additional substitution of serine by alanine at position 264 revealed that this mutant is insensitive to DCMU [9]. Therefore, all these

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 71, No 3, 2018

BIOLOGY
Biophysics

**IMPACT OF SALICYLIC ACID ON THE GROWTH
AND THE ACTIVITY OF PHOTOSYNTHETIC APPARATUS
IN RICE UNDER NON-STRESS CONDITIONS**

**Ekaterina Yotsova, Anelia Dobrikova, Martin Stefanov,
Emilia Apostolova**

(Submitted by Academician K. Koumanov on March 9, 2017)

Abstract

The impact of exogenous application of different concentrations of salicylic acid (10, 50 and 100 μM) through the rooting medium on the plant growth, the pigment content and the photochemical activities of both photosystem I and photosystem II was investigated. Data revealed that the observed alterations strongly depend on the concentration of applied salicylic acid, as 10 μM is the optimal concentration for the growth and the functional activity of photosynthetic apparatus of rice plants under non-stress conditions. In addition, the concentrations of salicylic acid lower than 100 μM had no effect on the energy transfer between the chlorophyll-protein complexes in thylakoid membranes.

Key words: rice, salicylic acid, photochemical activity, pigments, P700, 77K chlorophyll fluorescence

Improvement of the rice photosynthetic apparatus defence under cadmium stress modulated by salicylic acid supply to roots

Ekaterina K. Yotsova · Anelia G. Dobrikova · Martin A. Stefanov · Margarita Kouzmanova · Emilia L. Apostolova

Received: 5 December 2017 / Accepted: 26 February 2018 / Published online: 10 March 2018
© Brazilian Society of Plant Physiology 2018

Abstract The present study was conducted to investigate the effect of exogenous salicylic acid (SA) added to the nutrient solution on the growth parameters and the functions of the photosynthetic apparatus of rice plants under cadmium (Cd) stress. Our investigations have shown that 10 μM SA has an optimal effect in rice plants grown hydroponically. Pulse amplitude modulated chlorophyll fluorescence, low-temperature chlorophyll fluorescence, oxygen evolution (measured with Clark-type and Joliot-type electrodes) and P700 photo-oxidation measurements were carried out to assess the effect of SA on the activity of the photosynthetic apparatus. The levels of three important parameters associated with oxidative stress (hydrogen peroxide, lipid peroxidation and proline content) were measured. The application of low concentration of SA significantly decreased the levels of hydrogen peroxide, lipid peroxidation and proline under Cd stress. The results revealed that low concentration of SA, applied in plants exposed to 150 μM CdCl_2 , significantly improves plant growth, photochemical activities of both photosystems, the

electron flow from Q_A to plastoquinone, energetic distribution between pigment-protein complexes and the kinetic parameters of oxygen-evolving reactions. This study suggests that exogenous application of 10 μM SA through the rooting medium has a protective effect against Cd toxicity in rice plants. The possible molecular mechanisms involved in the defence effect of SA on the function of photosynthetic apparatus are discussed.

Keywords Cadmium stress · Chlorophyll fluorescence · Oxidative stress · Photochemical activity · Pigments · Rice · Salicylic acid

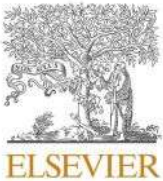
1 Introduction

Heavy metals are one of the major environmental pollutants, whose concentrations have been increasing continuously in the soil and in the water, and their quantities are often sufficient to present a risk to the human health. Plants grown in metal-polluted environments, exhibit altered metabolism, growth and biomass reduction, lower crop yields and metal accumulation (for review see Parmar et al. 2013; Tran and Popova 2013).

Cadmium (Cd) is one of the most toxic heavy metals because of its high solubility in water, its easy absorption through the roots and its accumulation in plant tissues (see Li et al. 2017). This metal strongly

E. K. Yotsova · A. G. Dobrikova · M. A. Stefanov · E. L. Apostolova (✉)
Institute of Biophysics and Biomedical Engineering,
Bulgarian Academy of Sciences, Sofia, Bulgaria
e-mail: emya@bio21.bas.bg

M. Kouzmanova
Department of Biophysics and Radiobiology, Biological
Faculty, Sofia University, Sofia, Bulgaria



Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy



Research article

Effects of cadmium on two wheat cultivars depending on different nitrogen supply



Ekaterina Yotsova^a, Anelia Dobrikova^a, Martin Stefanov^a, Svetlana Misheva^b,
Monika Bardáčová^c, Ildikó Matusšíková^c, Laura Žideková^d, Alžbeta Blehová^e,
Emília Apostolova^{a,*}

^a Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, 1113, Sofia, Bulgaria

^b Institute of Plant Physiology and Genetics, Bulgarian Academy of Science, 1113, Sofia, Bulgaria

^c University of SS. Cyril and Methodius, Faculty of Natural Sciences, Department of Ecochemistry and Radioecology, Námestie J. Herdu 2, Trnava, SK-917 01, Slovak Republic

^d University of SS. Cyril and Methodius, Department of Biochemistry, Námestie J. Herdu 2, Trnava, SK-917 01, Slovak Republic

^e Comenius University in Bratislava, Faculty of Natural Sciences, Department of Plant Physiology, Ilkovicova 6, Mlynská dolina, 84 215, Bratislava, Slovak Republic

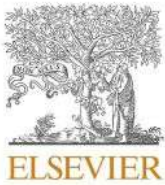
ARTICLE INFO

Keywords:

Photosynthetic apparatus
Cadmium
Nitrogen
Oxidative stress
Oxygen evolution
Pigments
Wheat cultivars

ABSTRACT

Heavy metal pollution as well as improper fertilization management represent serious threats to a clean environment and healthy food. This study was conducted to investigate how nitrogen supply influences a plant's ability to cope with cadmium stress in the two wheat cultivars – the modern cv. Katya (carrier of the semi-dwarfing gene *Rht8*) and the old cv. Slomer. Here we examined the effects of 100 μM CdCl_2 on both wheat genotypes grown hydroponically under three different nutrition regimes of 5.5, 10 and 20 mM NO_3^- by investigating plant growth, pigment content and the functional activity of the photosynthetic apparatus through a combination of PAM chlorophyll fluorescence, P700 photooxidation, oxygen evolution and oxidative stress markers. Data showed that the different genetic background affects the different strategies for metal uptake and allocation, as well as abilities to deal with oxidative stress. The modern cv. Katya restricts the entry of the metal to the roots, but allows its translocation to the shoots. Nevertheless, the photosynthetic performance indicated better protection, possibly mediated by the *Rht8* allele. In contrast, the old cv. Slomer tolerates higher cadmium levels in roots and possesses efficient barriers against its transfer to the shoots, but still showed more impaired photosynthetic activity. In general, the impact of cadmium on the photosynthetic apparatus was most deleterious under the lowest nitrogen concentration which was applied, while the highest nitrogen supply alleviated the negative effects of cadmium. The data suggest that the modern breeding allele (*Rht8*), as well as a better nutrition might contribute to the tolerance to heavy metal stress in the wheat.



Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy



Impact of foliar spray of zinc oxide nanoparticles on the photosynthesis of *Pisum sativum* L. under salt stress

Hisham A. Elshoky^{a,b,1}, Ekaterina Yotsova^{c,1}, Mohamed A. Farghali^{a,d}, Khaled Y. Farroh^{a,b},
Kh El-Sayed^{a,d}, Heba Elsayed Elzorkany^{a,b}, George Rashkov^c, Anelia Dobrikova^c,
Preslava Borisova^c, Martin Stefanov^c, Maha Anwar Ali^e, Emilia Apostolova^{c,*}

^a Nanotechnology and Advanced Materials Central Lab, Agricultural Research Center, Giza, Egypt

^b Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt

^c Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

^d Nanotechnology Research Center, British University in Egypt, Egypt

^e Biophysics Department, Faculty of Sciences, Cairo University, Giza, Egypt

ARTICLE INFO

Keywords:

ZnO and ZnO-Si nanoparticles
PAM chlorophyll fluorescence
P700
Photosynthetic pigments
Salt stress
Stomata structure

ABSTRACT

This study investigates the impacts of zinc oxide nanoparticles: bare (ZnO NPs) and ZnO NPs coated with silicon shell (ZnO-Si NPs), on *Pisum sativum* L. under physiological and salt stress conditions. The experimental results revealed that the foliar spray with ZnO-Si NPs and 200 mg/L ZnO NPs did not influence the stomata structure, the membrane integrity, and the functions of both photosystems under physiological conditions, while 400 mg/L ZnO-Si NPs had beneficial effects on the effective quantum yield of photosystem II (PSII) and the photochemistry of photosystem I (PSI). On the contrary, small phytotoxic effects were registered after spraying with 400 mg/L ZnO NPs accompanied by stimulation of the cyclic electron flow around PSI and an increase of the non-photochemical quenching (NPQ). The results also showed that both types of NPs (with exception of 400 mg/L ZnO NPs) decrease the negative effects of 100 mM NaCl on the photochemistry of PSI (P700 photooxidation) and PSII (qp, Fv/Fm, Fv/Fo, Φ_{PSII} , Φ_{exc}), as well as on the pigment content, stomata closure and membrane integrity. The protective effect was stronger after spraying with ZnO-Si NPs in comparison to ZnO NPs, which could be due to the presence of Si coating shell. The role of Si shell is discussed.



ARTICLE

Microalgae Improve the Photosynthetic Performance of Rice Seedlings (*Oryza sativa* L.) under Physiological Conditions and Cadmium Stress

Ekaterina Yotsova¹, Martin Stefanov¹, Georgi Rashkov¹, Margarita Kouzmanova², Anelia Dobrikova¹ and Emilia Apostolova^{1,*}

¹Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Department of Biophysics and Radiobiology, Biological Faculty, Sofia University, Sofia, Bulgaria

*Corresponding Author: Emilia Apostolova. Email: emya@bio21.bas.bg

Received: 30 November 2021 Accepted: 19 January 2022

ABSTRACT

The aim of this study was to assess the impact of the microalgae *Chlorella vulgaris* on the rice seedlings at physiological conditions and under cadmium (Cd) stress. We examined the effects of *C. vulgaris* in the nutrient solution on rice seedlings grown hydroponically in the presence and the absence of 150 μM CdCl_2 , using the low (77 K) temperature and pulse amplitude modulated (PAM) chlorophyll fluorescence, P700 photooxidation measurements, photochemical activities of both photosystems, kinetic parameters of oxygen evolution, oxidative stress markers (MDA, H_2O_2 and proline), pigment content, growth parameters and Cd accumulation. Data revealed that the application *C. vulgaris* not only stimulates growth and improves the functions of photosynthetic apparatus under physiological conditions, but also reduces the toxic effect of Cd on rice seedlings. Furthermore, the presence of the green microalgae in the nutrient solution of the rice seedlings during Cd exposure, significantly improved the growth, photochemical activities of both photosystems, the kinetic parameters of the oxygen-evolving reactions, pigment content and decreased lipid peroxidation, H_2O_2 and proline content. Data showed that the alleviation of Cd-induced effects in rice seedlings is a result of the Cd sorption by microalgae, as well as the reduced Cd accumulation in the roots and its translocation from the roots to the shoots.

KEYWORDS

Chlorella vulgaris; growth parameters; low temperature chlorophyll fluorescence; PAM chlorophyll fluorescence; photosynthesis; rice; stress markers