Theor. Exp. Plant Physiol. (2018) 30:153–163
https://doi.org/10.1007/s40626-018-0110-9

Polyamines and amino acids in triticale plants grown on humic acids enriched nutrient solution and treated with UV-B irradiation

Iskren Sergiev · Dessislava Todorova · Zornitsa Katerova · Ida Brambilla · Sergio Mapelli · Svetlana Simova

Received: 26 January 2018 / Accepted: 12 May 2018 / Published online: 17 May 2018

Abstract The effects of UV-B irradiation and Biomin (a natural substance extracted from coal with active ingredients of humic acids) on the content of endogenous polyamines spermine, spermidine and putrescine, and free amino acids in shoots and roots of young triticale seedlings were investigated. Biomin was added to the nutrient medium 3 days prior to UV-B irradiation. The seedlings were treated with 7.7 kJ m^{-2} day^{-1} UV-B light for 4 days. The exposure to UV-B increased total free amino acids, while Biomin application alone did not affect considerably their content. The treatment with UV-B or Biomin alone provoked augmentation of conjugated and bound polyamine (PA) fractions. Data suggest that Biomin alleviates the negative consequences of UV-B stress, manifested by the normalized amino acid and polyamine amounts in the UV-B + Biomin-treated plants. This study demonstrates the protective effect of Biomin on triticale plants against UV-B irradiation, which could be related to alterations in PAs and amino acids pools.

Keywords Amino acids · Humic acids · UV-B radiation · Polyamines · Proline · Triticale

I. Sergiev · D. Todorova (✉) · Z. Katerova
Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bldg. 21, 1113 Sofia, Bulgaria
e-mail: dessita@bio21.bas.bg

I. Brambilla · S. Mapelli
National Research Council, Institute of Agricultural Biology and Biotechnology, via Bassini 15, 20133 Milan, Italy

S. Simova
Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bldg. 9, 1113 Sofia, Bulgaria

1 Introduction

Solar UV irradiation is divided into three classes: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). As sunlight passes through the atmosphere all of the UV-C and most of the UV-B light are absorbed by ozone, water vapor and oxygen. Depletion of the ozone layer has been observed during the second half of the 20th century as a result of human activities. This led to a significant increase of the ultraviolet (UV) irradiation reaching the Earth's surface. After adopting the so called Montreal Protocol, the use of ozone destructing compounds was restricted worldwide, and the ozone layer started to rebuild again (Björn 2015). Although promising, this tendency is still not stable and could be reversed. The UV-B irradiation produces a number of harmful effects in plant cells such as damage to proteins, membrane phospholipids, and DNA (Teramura 1983; Teramura and Sullivan 1994; Quaite et al. 1992; Zlatev et al. 2012). Protective physiological responses in plants

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2015) 39: 1008-1013

doi:10.3906/bot-1502-14

A comparative analysis of membrane intactness and genome integrity in pea, barley, and wheat in response to UVC irradiation

Mariyana GEORGIEVA[1,*], **Ivelina NIKOLOVA**[1], **Georgi BONCHEV**[1], **Zornitsa KATEROVA**[2], **Dessislava TODOROVA**[2]
[1]Department of Molecular Genetics, Institute of Plant Physiology and Genetics, Bulgarian Academy of Science, Sofia, Bulgaria
[2]Department of Regulation of Plant Growth and Development, Institute of Plant Physiology and Genetics, Bulgarian Academy of Science, Sofia, Bulgaria

Received: 06.02.2015 • **Accepted/Published Online:** 22.09.2015 • **Printed:** 21.12.2015

Abstract: The maintenance of plant genome integrity plays a critical function in the processes of DNA replication, transcription, and repair. Short-wave UV radiation (UVC) is among the most harmful agents known to affect genome stability and to induce DNA damage, including double-strand breaks (DSBs). Most previous studies in plants addressed the effects of UVC radiation at the physiological level; however, little research effort has been put into genome sensitivity across different plant species. Here, we made use of the trypan blue exclusion test and neutral comet assay to assess nuclear membrane and genome integrity in response to UVC radiation in monocot and dicot plants. We found that UVC radiation substantially affects nuclear membranes and the level of DSBs in a dose-responsive manner. Furthermore, differential sensitivity across plant species was observed, with monocot plants being less vulnerable to DSBs. This allows us to speculate that plant species with larger genomes may better tolerate UVC radiation.

Key words: Ultraviolet radiation, genome integrity, trypan blue exclusion test, neutral comet assay, DNA double-strand breaks

1. Introduction

Plants are continuously exposed to solar radiation. Ultraviolet (UV) radiation, one of the components of sunlight, can be divided into three categories: long-wave UVA (315–400 nm), medium-wave UVB (280–315 nm), and short-wave UVC (100–280 nm). The ozone layer efficiently absorbs UV radiation up to about 310 nm as it shields all UVC and more than 95% of UVB. The most comprehensive data are available about the effect of UVA and UVB radiation on plants. The physiological and genetic response of plant cells to UVA radiation has been observed during stem extension, leaf development, and phototropism (Kunz et al., 2006). Most biological macromolecules are targets of UVB radiation. Alterations in important processes like photosynthesis, photomorphogenesis, seed germination, growth and development, and secondary metabolism have been observed (Mpoloka, 2008). Several studies reported an impact on membranes, phytohormones (Frohnmeyer and Staiger, 2003), and the activation of transposable elements (Qüesta et al., 2010).

UVC light is the most energetic and harmful photolytic agent that has the potential for inducing DNA damage, even at very short exposures. Similarly to UVB, the effects of UVC radiation on the plant genome can be of direct or indirect origin, detected mainly as pyrimidine dimers (adjacent thymine and cytosine), photoproducts (intrastrand cyclobutane-type pyrimidine dimers), which have the capacity to block DNA replication and transcription in plants cells. These lesions are repaired mainly by excision repair; however, incomplete processes can result in the formation of single-stranded DNA gaps sensitive to endonuclease attack (Myllyperkiö et al., 2000). Hence, DNA double-strand breaks (DSBs) also accumulate as a result of these described processes and are followed by chromosomal damage (Ma et al., 2009). In addition, UVC radiation contributes to the formation of DSBs in dividing cells most often through the production of intercellular reactive oxygen species (ROS) (Zemp et al., 2012). Several studies have reported the accumulation of endogenous DSBs caused by "cutting effects" or by the occurrence of a sufficient amount of adjacent single-strand breaks in human cells (Bogdanov et al., 1997; Tashiro, 2000). The effects of UVC irradiation on DNA depend on cell type and proliferation status, DNA repair capability, and the presence of endogenous and exogenous photosensitizers (Stapleton, 1992). Since monocotyledonous (monocot) plants have vertical patterns of leaf growth they tend to

* Correspondence: mimy80@abv.bg

Turkish Journal of Botany
http://journals.tubitak.gov.tr/botany/
Research Article

Turk J Bot
(2014) 38: 747-753

doi:10.3906/bot-1312-52

Biochemical responses of triticale plants treated with UV-B irradiation and nutrient solution enriched with humic acids

Dessislava TODOROVA[1,*], Iskren SERGIEV[1], Irina MOSKOVA[1], Zornitsa KATEROVA[1], Nina GEORGIEVA[1], Vera ALEXIEVA[1], Ida BRAMBILLA[2], Sergio MAPELLI[2]
[1]Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Agricultural Biology and Biotechnology, National Research Council, Milan, Italy

Received: 17.12.2013 • **Accepted:** 30.03.2014 • **Published Online:** 20.05.2014 • **Printed:** 19.06.2014

Abstract: A natural substance extracted from coal with humic acids as its active ingredients, namely Biomin, was added to nutrient medium and applied to triticale roots 3 days prior to UV-B irradiation treatment. UV-B treatment increased malondialdehyde and anthocyanin contents and the activities of peroxidase and superoxide dismutase, while it decreased chlorophyll content, fresh weight, and shoot length. Catalase and total phenolics content did not change in UV-B–treated shoots. The pretreatment with Biomin showed favorable effects on growth, decreased the oxidative damage provoked by UV-B irradiation, increased the content of UV-B–absorbing compounds, and positively influenced enzymatic activities. The application of Biomin on triticale plants was beneficial for counteracting the UV-B–induced oxidative stress by increasing the content of nonenzymatic antioxidants and antioxidant enzyme activities involved in detoxification of reactive oxygen species.

Key words: Defense enzymes, humic acids, Biomin, triticale, UV-B stress

1. Introduction

As a result of stratospheric ozone depletion, the amount of solar ultraviolet-B (UV-B) radiation reaching Earth has been increasing. Negative effects of increased UV-B irradiation on plant ecosystems are well known. UV-B modifies plant morphology, reduces growth, alters biosynthesis of secondary metabolites, induces oxidative stress via overproduction of reactive oxygen species (ROS), disturbs the normal physiological processes, and even leads to plant death (Frohnmeyer and Staiger, 2003; Bassman, 2004; Edreva, 2005). To minimize the detrimental effects of UV-B radiation, plants have evolved various detoxification mechanisms, such as enhancement of the antioxidant system (Brosché and Strid, 2003), induction of photolyases, and accumulation of UV-absorbing compounds (Frohnmeyer and Staiger, 2003; Fedina et al., 2007).

When the strength of the stressor pressure does not exceed the endogenous defense capacity, plants are able to overcome negative stress effects. The effectiveness of the antioxidant defense systems could be enhanced by application of compounds possessing different chemical natures or physiological modes of action. Applied in low doses, these substances could activate cell metabolism, improve plant physiological processes, and increase plant resistance to various unfavorable stress factors (Park et al., 2006; Todorova et al., 2008; Habibi, 2012; Kabiri et al., 2012; Pandey et al., 2012; Todorova et al., 2012).

Humic substances (HSs), including humic acids (HAs), are natural organic polyelectrolytes in the soil humus that stabilize the soil organic matter (Chen et al., 2004a). Several authors (Chen and Aviad, 1990; Chen et al., 2004a, 2004b; Mora et al., 2010) have reported the ability of HSs to increase the growth of different plant species grown under adverse conditions. However, the exact mechanism responsible for this effect of HS is poorly understood. Some authors suggested that HSs promote plant growth by improving the bioavailability of certain nutrients, mainly iron and zinc (Chen et al., 2004a, 2004b). Others proposed that HSs can directly influence the plant metabolism by both activating the root plasma membrane ATPase activity and increasing the nitrate uptake rates in roots (Nardi et al., 2002). This could act as a signal for root-to-shoot distribution of certain plant growth regulators (polyamines) and phytohormones (cytokinins and abscisic acid) (Mora et al., 2010). To our knowledge, limited information is available about the possibilities of HSs to protect plants grown under unfavorable conditions

* Correspondence: dessita@bio21.bas.bg

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 67, No 4, 2014

BIOLOGIE
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BIOCHEMICAL RESPONSES OF TWO TOMATO GENOTYPES DIFFERING IN GENE *ANTHOCYANINLESS OF HOFFMANN* (*AH*), TREATED WITH UV-B IRRADIATION AND β-MONOMETHYL ESTER OF ITACONIC ACID (MEIA)

Zornitsa Katerova, Elena Shopova, Tania Kartseva, Elena Balacheva, Dessislava Todorova

(*Submitted by Academician A. Atanassov on September 12, 2013*)

Abstract

Tomato (*Solanum lycopersicum*) – cv. Ailsa Craig (ACr, wild type) and its isogenic/near isogenic line [IL/NIL] *ah* (*anthocyaninless of Hoffmann*) were grown as a soil culture. Four-week-old plants were treated with 1mM β-monomethyl ester of itaconic acid (MEIA) and 24 h later were irradiated with 12.8 kJm^{-2}d^{-1} UV-B. Hydrogen peroxide (H_2O_2), free proline, free thiols and total phenols were measured in the fourth leaf of plants at 0, 24 and 48 h after cessation of UV-B irradiation. At the end of experiment all irradiated plants showed desiccation and curling of some leaf nodes. These negative effects were less expressed by application of MEIA prior to UV-B especially for ACr cv., containing anthocyanins. Concentration of H_2O_2 rise in UV-B treated plants but preliminary application of MEIA lessen this stress marker in ACr cv. whereas in anthocyaninless mutant it was permanently enhanced. Combined treatment provoked permanently augmented proline levels in both lines, with exception of data for anthocyaninless mutant at 24 h after irradiation. Preliminary application of MEIA also led to lower accumulation of free thiols and total phenolics as compared to irradiated only plants especially in ACr cv. Anthocyaninless mutant is more sensitive to UV-B stress than the wild type and possesses less total phenolic compounds, compensated by higher concentrations of free thiols measured at 24 and 48 h in combined variant. Comparative data analyses of phenotypic effects and non-enzymatic antioxidant's amount

This study was supported by project DMU 03/60.

suggest that MEIA has protective effect against UV-B irradiation through activation of different defence mechanisms related to particular characteristics of both tomato genotypes, and this effect was more pronounced for anthocyanins containing genotype.

Key words: antioxidants, hydrogen peroxide, protector, tomato, UV-B radiation

Abbreviations: ACr – Ailsa Craig, wild type, *ah – anthocyaninless of Hoffmann* mutant line, GAE – gallic acid equivalents, H_2O_2 – hydrogen peroxide, MEIA – β-monomethyl ester of itaconic acid, ROS – reactive oxygen species, UV – ultraviolet radiation

Introduction. During the past few decades a decrease of ozone layer as a result from human activities has been observed. The reduction of the ozone layer could lead to a significant increase of UV-B irradiation (290–320 nm). UV-B irradiation has a range of negative effects on plant organisms: it reduces growth and alters morphology, disrupts important macromolecules, modifies biosynthesis of secondary metabolites, provokes oxidative stress via overproduction of reactive oxygen species, disturbs the normal physiological processes and may even cause death [1, 2]. To reduce the noxious effects of UV-B radiation, plants have developed a variety of detoxification mechanisms, such as enhancement of the antioxidant system, activation of photolyases and accumulation of UV-absorbing compounds [1–3]. Plants are able to overcome the harmful stress effects by themselves when the strength of the stressor does not exceed the endogenous defence capacity. Application of compounds possessing different chemical nature or physiological mode of action could enhance the effectiveness of the antioxidant defence systems when the strength of the stressor exceeds the plant protection capacity. When applied in low doses, the substances activate cell metabolism, improve plant physiological processes, and increase plant resistance to various unfavorable stress factors [4–6]. It was previously shown that the β-monomethyl ester of itaconic acid, MEIA (derivative of naturally occurring dicarboxylic acid) had defensive effect against the herbicide chlorsulfuron in maize [7], UV-C radiation in wheat [8] and biotic stress in tomatoes [9]. Contemporary researches show that anthocyanins play a certain role in tolerance to stressors as diverse as drought, UV-B, and heavy metals, as well as resistance to herbivores and pathogens [3]. Nine mutations that result in the complete absence of anthocyanin in all plant organs during the whole vegetation period are known in tomato [10]. One of them, mutation *ah (Hoffmann's anthocyaninless)* was characterised by co-ordinate reduction in the activities of dihydroflavonol 4-reductase, chalcone synthase and flavone 3 hydroxylase – the key enzymes involved in phenolic secondary metabolites [11]. The plants have ability to synthesise flavones and/or flavonols, but not anthocyanins.

The aim of the current study is to evaluate whether the protective effect of MEIA against UV-B irradiation varies within tomato genotype which contains

POLYAMINE SPERMINE PROTECTS YOUNG PEA PLANTS AGAINST ULTRAVIOLET-C RADIATION

Dessislava Todorova[1], Zornitsa Katerova[1], Elena Shopova[1], Asya Nikolova[1], Nina Georgieva[1], Iskren Sergiev[1], Sergio Mapelli[2]
[1]Bulgarian Academy of Sciences, Institute of Plant Physiology and Genetics, Sofia, Bulgaria
[2]National Research Council (CNR), Institute of Agricultural Biology and Biotechnology, Milan, Italy
Corresponding author: Dessislava Todorova
E-mail: dessita@bio21.bas.bg

ABSTRACT

The effects of ultraviolet-C (UV-C) irradiation and polyamine spermine on the content of some stress marker and non-enzymatic antioxidants in leaves of young pea plants were investigated. UV-C irradiation led to a decrease in pea fresh weight, the content of leaf pigments and free proline, accompanied with an increase in malondialdehyde. The initial augmentation in the free thiol levels was transient in UV-C treated plants and finally a substantial decrease was found. Spermine led to a significant augmentation of free thiols and proline content along with a decline in total phenols, but these alterations diminished during the experimental period. Based on comparative analyses of the results obtained for plants treated with UV-C and polyamine, it could be concluded that preliminary application of spermine protects pea plants against irradiation, by maintaining normal plant growth, stabilizing cell membranes and activating non-enzymatic antioxidants.

Biotechnol. & Biotechnol. Eq. 2013, **27**(3), 3798-3802

Keywords: oxidative stress, pea, plant protector, spermine, UV-C

Abbreviations: FW: fresh weight; MDA: malondialdehyde; PA: polyamine; ROS: reactive oxygen species; Spm: spermine; UV: ultraviolet

Introduction

Usually plants are subjected to various adverse environmental impacts (stressors), leading to induction of oxidative stress. Plants are able to cope with it by enzymatic and/or non-enzymatic protective systems, if the strength of the stressor does not exceed the capacity of this protection. Increasing the effectiveness of antioxidant protection may be achieved with application of exogenous substances (such as some plant growth regulators) of different chemical nature and physiological action. Applied at low doses, they can activate cellular metabolism and increase the physiological activity of plants to overcome the negative effects of a stressor.

Ultraviolet radiation (UV) is an abiotic stress factor. According to the International Commission on Illumination, the wavelengths between 400 nm and 200 nm are divided into three regions: UV-A (315 nm to 400 nm), UV-B (280 nm to 315 nm) and UV-C (200 nm to 280 nm). High UV doses lead to overproduction of different reactive oxygen species (ROS) and development of oxidative stress, which affects important plant processes (21, 33) and may decrease cell viability and even lead to cell death (29). ROS interact with macromolecules causing damage in nucleic acids, proteins and lipids. Investigations on ultraviolet radiation-triggered effects on plants have considered predominantly UV-B radiation but the damaging effect increases towards shorter wavelengths, UV-C being the most detrimental for organisms because of its highest energy (24). Although, UV-C also causes photochemical reactions in molecules of high biological significance and other types of damage in a similar way to UV-B, it seems that these light wave bands have different action mechanisms in plants (14, 24).

Polyamines are low-molecular-weight organic compounds and are positively charged under physiological pH conditions, which allows them to conjugate with other negatively charged organic molecules like phenolic acids, proteins, phospholipids or nucleic acids and to take part in essential growth and developmental plant processes. As a result of their polycationic nature, polyamines possess free radical scavenging properties, antioxidant activity and could affect plant tolerance to different biotic and abiotic stresses (9, 10). The most widespread polyamines in plants are the diamine putrescine, the triamine spermidine and tetraamine spermine (Spm). It is believed that Spm possesses the highest protective role against unfavorable conditions because of its highest positive charge due to its four amine groups (5). The aim of the present study was to evaluate the capabiltiy of Spm to act as a protector of pea plants irradiated with UV-C.

Materials and Methods

Model system

Young pea plants (*Pisum sativum* L., cv. Ran 1) were grown as water culture in a growth chamber (12 h/12 h photoperiod; 60 % to 70 % relative air humidity, 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photon flux density; 24 ºC ± 2 ºC). Thirteen-day- old seedlings were leaf sprayed with 1 $mmol \cdot L^{-1}$ Spm, and 24 h later pea plants were irradiated with UV-C for 30 min (9 $kJ \cdot m^{-2}$).

Germicidal lamp (STYLO STY 115, GE Lighting, Italy, λmax 254 nm) was used to supply the UV-C radiation, which

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

Tome 64, No 11, 2011

BIOLOGIE

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EFFECT OF ENHANCED UV-C IRRADIATION ON THE GROWTH, MALONDIALDEHYDE, HYDROGEN PEROXIDE, FREE PROLINE, POLYAMINES, IAA AND IAA-OXIDASE ACTIVITY IN PEA PLANTS (*PISUM SATIVUM* L.)

Zornitsa Katerova, Dessislava Todorova

(*Submitted by Academician A. Atanassov on June 20, 2011*)

Abstract

The effect of low dose (LD) of 0.1 kJ m^{-2} d^{-1} and high dose (HD) of 0.3 kJ m^{-2} d^{-1} of UV-C irradiation on some growth parameters (aboveground length, fresh and dry weight), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), free proline, free, conjugated and bound spermine (Spm), spermidine (Spd) and putrescine (Put), free indole-3-acetic acid (IAA) content and IAA-oxidase activity in leaves of pea plants after 21 days of consecutive treatment was studied. As it was expected all measured growth parameters were reduced in dose-dependent manner. LD regime does not influence stress markers content. HD regime led to insignificant change in proline amount, some increase in H_2O_2 content (assuming signal role), and considerable decrease in MDA amount. UV-C treatments cause substantial increase of bound PA-fractions, which correlated negatively with reduced MDA and indicate lower lipid peroxidation of unsaturated fatty acids of biomembranes. A reduction of free PAs (except Spm) was found after both UV-C regimes. Drop in conjugated PAs concentration was observed only in Spd after LD regime. Alterations in free and conjugated PAs pool could be due to conversion of free into conjugated form. It was determined that HD regime lessen IAA content, while LD regime had an opposite effect. Possibly IAA changed specifically to different doses

Detection of IAA was supported by a grant from the Interuniversity Poles of Attraction Programme (Belgian State, Prime Minister's Office – Federal Office for Scientific, Technical and Cultural Affairs; IUAP-V P5/13) and by specialization fellowship No 13DA-Iz/JDW/BUL/2003-2004/03-2250 from the bilateral cultural programme between the Ministries of Bulgarian and Flemish Community.

of UV-C irradiation without participation of IAA-oxidase as its activity was insignificantly altered.

This study reinforces previous reports with the same model system and shorter treatment periods (7 and 14 d LD and HD). On the basis of the presented here results we assume that the additional period of UV-C irradiation facilitates pea to improve repair processes under prolonged low intensive UV-C treatment.

Key words: UV-C radiation, pea, polyamines, IAA, IAA-oxidase, hydrogen peroxide, malondialdehyde, proline

Abbreviations: HD – high dose UV-C, H_2O_2 – hydrogen peroxide, IAA – indole-3-acetic acid, LD – low dose UV-C, MDA – malondialdehyde, PAs – polyamines, Put – putrescine, Spd – spermidine, Spm – spermine, UV – ultraviolet radiation

Introduction. Ultraviolet radiation is classified as UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). UV-C quickly creates high levels of damage, more than UV-B, because of its highest energy [1]. Generally, UV-C irradiation does not reach the Earth's surface due to its absorption in the atmosphere, with the exception of high mountain locations [2]. In general, studies on ultraviolet radiation-induced effects in plants have considered mainly UV-B exposure, but either UV-B or UV-C photons possess enough energy to destroy chemical bonds causing a photochemical reaction. The overproduction of different reactive oxygen species (hydrogen peroxide, singlet oxygen, superoxide, and hydroxyl radicals) and development of oxidative stress affect various plant processes in UV-C irradiated plants. The effects have been classified into two categories: DNA injuries and damage to physiological processes [1]. Plants possess different defence systems to overcome harmful stress consequences, including various antioxidant enzymes (like superoxide dismutase, catalase, peroxidase, etc.), non-enzymatic compounds (i.e. proline, ascorbic acid, glutathione, phenolic acids, thiols, etc.), as well as plant growth regulators and phytohormones (as polyamines and cytokinins). Auxins as indole-3-acetic acid are involved mainly in normal developmental processes (growth, apical dominance, lateral root initiation) but they are also important regulators of plant responses to abiotic stresses [3]. The main polyamines (PAs) putrescine (Put), spermidine (Spd) and spermine (Spm) are aliphatic nitrogen-containing compounds positively charged at physiological pH that allows interacting with negatively charged groups of small molecules (like phenolic acids) or macromolecules as DNA and RNA, proteins and phospholipids. Thus, they are involved in the control of various cellular functions: antioxidant activity, scavenging of reactive oxygen species, and modulation of plant stress tolerance [4].

We have already published few articles concerning the alteration of some stress markers [5,6], IAA [7] and polyamines [8] in pea plants exposed to 7, 10 and/or 14 days of low intensive UV-C radiations. Here, we report the changes

Plant Secondary Metabolites and Some Plant Growth Regulators Elicited by UV Irradiation, Light And/Or Shade

Zornitsa Katerova, Dessislava Todorova and Iskren Sergiev

Abstract Classification of plant secondary metabolites and characterization of major plant growth regulators are shortly described. A short account is also given to light, shade and ultraviolet radiation and their impact on plants. Recent investigations regarding secondary metabolite production and alterations in endogenous level of plant growth regulators in medicinal plants grown under light, shade or UV radiation are reviewed and discussed. Some conclusions and future perspectives to enlarge the investigations in this direction are also given.

Keywords Light · Plant growth regulators · Plant secondary metabolites · Shade · UV-irradiation

Abbreviations

ABA	Abscisic acid
CK	Cytokinin
FR	Far red light
GA	Gibberellic acid
IAA	Indole-3-yl acetic acid
PAR	Photosynthetically active radiation
R	Red light
ROS	Reactive oxygen species
SAR	Shade avoidance response
UV	Ultraviolet radiation

Z. Katerova (✉) · D. Todorova · I. Sergiev
Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences,
Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
e-mail: zkaterova.landzhova@gmail.com

M. Ghorbanpour and A. Varma (eds.), *Medicinal Plants and Environmental Challenges*, https://doi.org/10.1007/978-3-319-68717-9_6

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

Tome 65, No 4, 2012

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POLYAMINES AND FREE PROLINE PROTECT YOUNG PEA (*PISUM SATIVUM* L.) LEAVES AGAINST ENHANCED UV-C IRRADIATION

Zornitsa Katerova, Dessislava Todorova

(*Submitted by Academician A. Atanassov on November 21, 2011*)

Abstract

Pea (*Pisum sativum* L.) seedlings were exposed to short pulses of ultraviolet-C (UV-C) radiations: low dose (LD – 0.1 kJ m^{-2} d^{-1}) and high dose (HD – 0.3 kJ m^{-2} d^{-1}). Concentrations of malondialdehyde (MDA); hydrogen peroxide (H_2O_2); free proline; free, conjugated and bound spermine (Spm), spermidine (Spd) and putrescine (Put) were determined in 4th leaves of pea plants after 21 consecutive days of UV-C treatment. Free proline, H_2O_2 and MDA did not change markedly after LD regime. HD regime led to a significant increase in free proline and H_2O_2 amount, accompanied by a considerable decrease in MDA content. HD treatment causes a substantial increase in bound PA-fractions. Any significant changes were found in bound fraction after LD regime. Alterations in free and conjugated PAs pool provoked by both UV-C treatments could be due to conversion of free into conjugated form and vice versa. HD regime provokes adaptation processes in 4th leaves: the augmentation of free proline and bound PAs protects cell membranes against prolonged low intensive UV-C radiation. This confirms that endogenous PAs as well as proline have an important role in plant defence responses under short pulses of UV-C treatment.

Key words: UV-C radiation, pea, polyamines, hydrogen peroxide, malondialdehyde, proline

Abbreviations: HD – high dose UV-C, H_2O_2 – hydrogen peroxide, LD – low dose UV-C, MDA – malondialdehyde, PAs – polyamines, Put – putrescine, ROS – reactive oxygen species, Spd – spermidine, Spm – spermine, UV – ultraviolet radiation

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Tome 65, No 10, 2012

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MEIA ACTS AS PROTECTOR AGAINST UV-C IRRADIATION IN YOUNG WHEAT PLANTS

Zornitsa Katerova, Elena Shopova, Nina Georgieva, Asya Nikolova, Iskren Sergiev, Dessislava Todorova

(*Submitted by Academician A. Atanassov on April 19, 2012*)

Abstract

Young wheat plants (*Triticum aestivum* L., cv. Sadovo 1) grown as water culture were treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and 24 h later were irradiated with 0.75 kJ m^{-2} day^{-1} of UV-C light for 5 consecutive days. Twenty hours after the cessation of the stress programme, the amount of malondialdehyde (MDA), hydrogen peroxide, free proline, free thiols and total phenols was measured in the first leaf of plants. All measured parameters were increased by UV-C irradiation as compared to relative control values. Application of MEIA prior to UV-C led to reduction in stress marker contents (MDA and free proline) accompanied with an additional increase in the amount of low-molecular thiols and total phenols (measured as part of non-enzymatic antioxidant defence system) as compared to these measured in plants treated only with UV-C. Data obtained suggest that MEIA protects young wheat plants against UV-C irradiation.

Key words: hydrogen peroxide, malondialdehyde, free proline, protector, thiols, total phenols, UV-C radiation, wheat

Abbreviations: GAE – gallic acid equivalents; H_2O_2 – hydrogen peroxide; MDA – malondialdehyde; MEIA - β-monomethyl ester of itaconic acid; ROS – reactive oxygen species; UV – ultraviolet radiation

Introduction. The three major classes of ultraviolet (UV) radiation are UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). Except for high mountain locations UV-C irradiation does not reach the Earth's surface due to its absorption in the atmosphere [1]. Importantly, UV-C is most detrimental

This study was supported by project DMU 03/60.

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

Tome 69, No 1, 2016

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BIOCHEMICAL RESPONSES OF YOUNG WHEAT PLANTS IRRADIATED WITH UV-C AND PRETREATED WITH β-MONOMETHYL ESTER OF ITACONIC ACID (MEIA) OR POLYAMINE SPERMINE

Zornitsa Katerova, Dessislava Todorova, Iskren Sergiev, Chih-Wen Yu*, Vera Alexieva

(*Submitted by Academician A. Atanassov on October 27, 2015*)

Abstract

Twelve-days-old wheat plants (*Triticum aestivum* L., cv. Sadovo-1) grown as water culture in growth chamber were treated with 1 mM MEIA or 1 mM spermine and 24 h later were subjected for 60 min to UV-C irradiation (18 kJ m^{-2} day^{-1}). Plant responses to the treatments were determined at 0 and 48 h after cessation of the stress. The alterations of free proline, malondialdehyde, low-molecular thiols, and total phenols suggested that pretreatment with MEIA and spermine mitigated the harmful effect of UV-C irradiation by decreasing the level of stress markers and increasing the content of thiol-containing antioxidants in wheat plants.

Key words: antioxidants, β-monomethyl ester of itaconic acid, spermine, UV-C irradiation, wheat

Abbreviations: FW – fresh weight, MDA – malondialdehyde, MEIA – β-monomethyl ester of itaconic acid, ROS – reactive oxygen species; Spm – spermine, UV – ultraviolet radiation

Introduction. According to the International Commission on Illumination the wavelengths between 400 and 200 nm are divided into three regions: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). The negative effect of UV radiation increases towards the shorter wavelengths, and UV-C irradiation is most detrimental for live organisms because of its highest energy [1]. High UV-C doses cause photochemical reactions in the living cells and overproduction of a variety of reactive oxygen species (ROS), which in turn provoke development

The authors express their gratitude to project DMU 03/60 and to Bulgaria-Taiwan joint research project (2014–2015).

10.1515/botlit-2017-0010

DE GRUYTER OPEN

BOTANICA LITHUANICA
ISSN 2029-932X

2017, 23(2): 79–88

AUXIN-LIKE COMPOUNDS ACT AS PROTECTORS AGAINST UV-B IRRADIATION IN GARDEN PEA PLANTS

Iskren Sergiev[1], Dessislava Todorova[1*], Elena Shopova[1], Zornitsa Katerova[1], Jurga Jankauskienė[2], Sigita Jurkonienė[2]

[1]Bulgarian Academy of Sciences, Institute of Plant Physiology and Genetics, Acad. G. Bonchev Str. Bl. 21, Sofia BG-1113, Bulgaria
[2]Nature Research Centre, Institute of Botany, Akademijos Str. 2, Vilnius LT-08412, Lithuania
*Corresponding author. E-mail: dessita@bio21.bas.bg

Abstract

Sergiev I., Todorova D., Shopova E., Katerova Z., Jankauskienė J., Jurkonienė S., 2017: Auxin-like compounds act as protectors against UV-B irradiation in garden pea plants. – Bot. Lith., 23(2): 79–88.

Pretreatment with the original auxin physiological analogues 1-[2-chloroethoxycarbonylmethyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylaminoethoxicarbonylmethyl]naphthalene chlormethylate (TA-14) and subsequent UV-B irradiation (180 min at λ_{max} 312 nm for 6.6 kJ·m^{-2}) of pea plants (*Pisum sativum* L.) was investigated to assess if foliar application of these compounds has ability to attenuate the negative effects caused by UV-B stress. UV-B treatment increased malondialdehyde (MDA) and proline levels as well as superoxide dismutase, catalase and guaiacol peroxidase activities, but decreased hydrogen peroxide, low-molecular thiols, total phenolics and total soluble protein contents. The pre-treatment with TA compounds decreased the oxidative stress provoked by UV-B radiation detected by lower level of MDA, increased the content of thiols and UV-absorbing compounds and had favourable effect on H_2O_2 content and enzymatic activities. Exogenous application of auxin-like compounds on pea plantlets successfully counteracted UV-B induced oxidative stress *via* activation of ROS detoxifying enzymes and non-enzymatic antioxidants.

Keywords: auxin-like compounds, antioxidants, pea, plant stress, stress markers, UV-B.

INTRODUCTION

Solar UV radiation is divided into three classes: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). As sunlight passes through the atmosphere, all UV-C and most UV-B are absorbed by ozone, water vapour and oxygen. Depletion of ozone layer due to the human activities is one of the causes that increase UV-B irradiation on Earth surface. The UV-B radiation produces a number of harmful effects in plant cells such as damage to proteins, membrane phospholipids, and DNA (Zlatev et al., 2012). To defeat from ultraviolet radiation, protective physiological responses in plants might be activated, including changes in antioxidant enzyme activities, non-enzymatic antioxidants, secondary metabolites, etc. (Gill & Tuteja, 2010; Zlatev et al., 2012). Exogenous application of different plant growth-regulating substances is also able to activate some or all of these defence systems in plants subjected to different type of abiotic stresses (Todorova et al., 2008; Ding et al., 2010; Habibi, 2012; Katerova et al., 2014; Todorova et al., 2014; Esringu et al., 2016; Sergiev et al., 2016; Aksakal et al., 2017).

Auxins are major class of plant hormones that positively influence plant growth and development processes such as cell division and enlargement, root initiation, buds formation, growth of root stem apexes as well as contribute to plant phototropism, geotropism and hydrotropism. They are also involved in plant adaptive stress responses to different stresses (Kazan, 2013), including UV-B irradiation (Van-

Chapter 6
Glutathione and Herbicide Resistance in Plants

Zornitsa Ivanova Katerova and Lyuba Petar-Emil Miteva

Abstract Pesticide use is inseparable part of food production. The efficacy of modern agriculture is quite dependent on the chemicals used to fight with pests, including weeds, fungi and insects. Herbicides are chemicals which destroy weeds. According to their mode of action herbicides are divided on 24 groups (Herbicide Resistance Action Committee). The balance between toxicity on weeds and resistance of crops defines herbicide selectivity. Herbicide tolerance depends on the plants variety, development phase, climate, mode of action, dose and the way plants were treated with herbicides. Glutathione is one of the major defense substances of plants. It takes part in many detoxifying mechanisms, like active oxygen species reducing, and also regulates cell defense systems. Glutathione has key role in detoxifying of toxic xenobiotics, including herbicides. In some crops the resistance against herbicides is due to its direct detoxification by forming conjugates with glutathione. The process can be catalyzed by the enzyme glutathione S-transferase. After their forming, conjugates can be metabolized and excreted or can be stored in vacuoles and dead tissues. Many herbicides, such as atrazine, paraquat, etc., induce oxidative events in plant cell. Glutathione takes part in detoxifying active oxygen species and this is a way for indirect enhancement of plant resistance against herbicides. In the current review the mode of action of herbicides inducing oxidative stress will be discussed. Examples of plant antioxidant system response against herbicide action will be presented. The role of glutathione in direct and indirect detoxification of herbicides and increase of plants sustainability will be deeply reviewed.

Keywords Glutathione • Herbicides • Herbicide resistance

Z.I. Katerova (✉)
Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
e-mail: katerovazor@yahoo.com

L.P.-E. Miteva
Resbiomed EOOD, 81 B, Bulgaria Blvd, Sofia, Bulgaria

N.A. Anjum et al. (eds.), *Ascorbate-Glutathione Pathway and Stress Tolerance in Plants*, DOI 10.1007/978-90-481-9404-9_6, plants MDPI

Article

Physiological Responses of Wheat Seedlings to Soil Waterlogging Applied after Treatment with Selective Herbicide

Zornitsa Katerova, Iskren Sergiev *, Dessislava Todorova, Elena Shopova, Ljudmila Dimitrova and Liliana Brankova

Department Regulators of Plant Growth and Development, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria; zkaterova.landzhova@gmail.com (Z.K.); dessita@bio21.bas.bg (D.T.); kostei@abv.bg (E.S.); dim.lyudmila@gmail.com (L.D.); lbrankova@abv.bg (L.B.)
* Correspondence: iskren@bio21.bas.bg

Abstract: Waterlogging impairs crop development and considerably affects plant productivity worldwide. Wheat is sensitive to waterlogging. Serrate® (Syngenta) is a selective herbicide controlling annual grass and broadleaf weeds for use in wheat. To extend the existing information about the physiological effects of selective herbicides (Serrate® in particular) and subsequent waterlogging in wheat, we monitored phenotype alterations and examined key enzymatic and non-enzymatic antioxidant defense systems together with typical oxidative stress biomarkers. Seventeen-day-old wheat (*Triticum asetivum* L., cv. Sadovo-1) plants were sprayed with Serrate®; 72 h later, waterlogging was applied for 7 days, and then seedlings were left to recover for 96 h. The herbicide did not alter plant phenotype and increased antioxidant defense, along with H_2O_2 content, confirming the wheat's tolerance to Serrate®. Evident yellowing and wilting of the leaves were observed at 96 h of recovery in waterlogged wheat, which were stronger in plants subjected to Serrate® + waterlogging. Waterlogging alone and herbicide + waterlogging gradually enhanced the content of stress markers (malondialdehyde, proline, and H_2O_2), non-enzymatic antioxidants (low-molecular thiols and total phenolics), and the activity of superoxide dismutase, guaiacol peroxidase, and glutathione reductase. The effects of herbicide + waterlogging were stronger than those of waterlogging alone even during recovery, suggesting that Serrate® interacted synergistically with the subsequently applied flooding.

Keywords: antioxidants; herbicide; stress markers; waterlogging; wheat

Citation: Katerova, Z.; Sergiev, I.; Todorova, D.; Shopova, E.; Dimitrova, L.; Brankova, L. Physiological Responses of Wheat Seedlings to Soil Waterlogging Applied after Treatment with Selective Herbicide. *Plants* **2021**, *10*, 1195. https://doi.org/10.3390/plants10061195

Academic Editor: Marija Viljevac Vuletić

Received: 27 May 2021
Accepted: 9 June 2021
Published: 11 June 2021

1. Introduction

Different climate models predict that negative environmental alterations will expand due to climate change driven by pollution and global warming, and the reductions in plant productivity will become much more noticeable [1–4]. Waterlogging is a substantial obstacle to sustainable agriculture and is expected to increase due to climate change. It can be a result of soil erosion; bad soil drainage; or unexpected, sudden, and heavy rainfall leading to floods [5]. Excess water negatively affects plant growth and development, leading to sizable yield losses. Waterlogging causes a number of changes in important soil physiochemical properties such as pH, redox potential, and oxygen access. Thus, plants grown on waterlogged soil are subject to adverse growth and negative developmental conditions such as hypoxia (O_2 insufficiency) or anoxia (lack of O_2), inhibition of aerobic respiration, energy deprivation, and oxidative stress. These conditions trigger an over-accumulation of reactive oxygen species (ROS), which impede plant growth, leading to senescence and cell death. In wheat production, significant harvest losses due to waterlogging were estimated to be 15–20% on an annual basis [6–9].

Another major problem in crop cultivation is spontaneous weed growth. The necessity for control of wild plants in arable fields led to the development of different chemicals to selectively demolish weeds, increase crop yields, and economize human resources and time.

412 nm [45]. The total content of phenolic compounds was measured according to Swain and Goldstein [46]. The reaction mixture was incubated for 2 h at room temperature, then the absorbance was read at 725 nm and the content of total phenolics was calculated by a standard curve prepared with gallic acid.

The activity of antioxidant enzymes was measured in supernatant obtained from approximately 200 mg of leaf material after grinding in 3 mL of cold 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% PVP and centrifugation at 15,000× g (4 °C). The activity of guaiacol peroxidase (EC 1.11.1.7) was measured using 1% guaiacol as an electron donor and 15% H_2O_2 as a substrate. The change in the absorbance was monitored for 1 min at 470 nm [47]. Catalase activity (EC 1.11.1.6) was measured by following the decomposition of 6% H_2O_2 for 1 min at 412 nm [48]. Glutathione reductase activity was measured by monitoring the reduction of GSSG for 1 min at 412 nm [49]. Superoxide dismutase activity was determined by the rate of inhibition of the photochemical reduction of nitroblue tetrazolium. The reaction was monitored at 560 nm. One unit of SOD defined the amount of enzyme needed to cause 50% inhibition [50]. The content of soluble protein was determined with Bradford's reagent [51].

The chemicals used in the analyses were purchased from local representative of Sigma-Aldrich, (Saint Louis, MO, USA) Serrate® was purchased from a local representative of Syngenta (Basel, Switzerland). Spectrophotometric measurements were conducted using Multiskan Spectrum (Thermo Electron Corporation, Uusimaa, Finland) and Shimadzu UV-1601 (Shimadzu, Kyoto, Japan) spectrophotometers. Supernatants were centrifuged in a refrigerated Sigma 2-16K centrifuge (SciQuip, Newtown, UK).

4.3. Statistics

The experiments were conducted three times. Samples for analyses were collected in three replicates. The data are presented as mean values ± SE. Duncan's multiple-range test was used to assess the significant differences between treatments at $p < 0.05$.

5. Conclusions

Serrate® application increased antioxidant defense and did not worsen phenotype plant traits, suggesting wheat adaptation. Waterlogging gradually enhanced the stress markers' content, had lag-time in antioxidant (enzymatic and non-enzymatic) induction, and wheat phenotype traits did not improve after the recovery period. Serrate® application induced a synergistic response in wheat subjected to waterlogging by aggravating the phenotypic traits of plants and did not recover successfully after cessation of the stress program; therefore, a monitoring forecast for flooding is recommended before Serrate® application to wheat as it may be unable to recover.

Author Contributions: I.S. and D.T., conceptualized and coordinated the research; L.D., Z.K. and D.T., grew and treated the plants; I.S., D.T., E.S., L.B. and Z.K., performed the laboratory analyses, collected and interpreted the data; I.S., prepared figures and photos; D.T. and Z.K., prepared original draft of manuscript; I.S. and D.T., reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Bulgarian National Scientific Fund (BNSF) grant KP-06-N36/3 (30 September 2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Assessment of the Biochemical Responses of Wheat Seedlings to Soil Drought after Application of Selective Herbicide

Dessislava Todorova, Iskren Sergiev *, Zornitsa Katerova, Elena Shopova, Ljudmila Dimitrova and Liliana Brankova

Institute of Plant Physiology and Genetics—Bulgarian Academy of Sciences, Acad G. Bonchev Str., Bl. 21. 1113 Sofia, Bulgaria; dessita@bio21.bas.bg (D.T.); zkaterova.landzhova@gmail.com (Z.K.); kostei@abv.bg (E.S.); dim.lyudmila@gmail.com (L.D.); lbrankova@abv.bg (L.B.)
* Correspondence: iskren@bio21.bas.bg

Abstract: Drought is a major environmental constrain with a deleterious effect on plant development leading to a considerable reduction of crop productivity worldwide. Wheat is a relatively drought tolerant crop during the vegetative stage. The herbicide Serrate® (Syngenta) is a preparation containing two active chemical substances with different modes of action, which inhibit the biosynthesis of fatty and amino acids. It is commonly used as a systemic and selective chemical agent to control annual grass and broadleaf weeds in cereal crops and particularly in wheat, which is tolerant to Serrate®. Seventeen-day-old wheat seedlings (*Triticum aestivum* L., cv. Sadovo-1) grown as soil culture under controlled conditions were sprayed with an aqueous solution of Serrate®. Seventy-two hours later the plantlets were subjected to drought stress for seven days to reach a severe water deficit followed by four days of recovery with a normal irrigation regime. Oxidative stress markers, non-enzymatic, and enzymatic antioxidants were analyzed in the leaves of plants from the different treatment groups (herbicide-treated, droughts-stressed, and individuals which were consecutively subjected to both treatments) at 0, 96, and 168 h of drought stress, and after 96 h of recovery. Herbicide treatment did not alter substantially the phenotype and growth parameters of the above-ground plant parts. It provoked a moderate increase in phenolics, thiol-containing compounds, catalase, superoxide dismutase, glutathione reductase, and H_2O_2. However, significant variations of malondialdehyde, proline, and peroxidase activity caused by the sole application of the herbicide were not detected during the experimental period. Drought and herbicide + drought treatments caused significant growth inhibition, increased oxidative stress markers, and activation of enzymatic and non-enzymatic antioxidant defense reaching the highest levels at 168 h of stress. Plant growth was restored after 96 h of recovery and the levels of the monitored biochemical parameters showed a substantial decline. The herbicide provoked an extra load of oxidative stress-related biochemical components which did not aggravate the phenotypic and growth traits of plants subjected to drought, since they exhibited a good physiological status upon recovery.

Keywords: antioxidants; drought; herbicide; stress markers; wheat

Citation: Todorova, D.; Sergiev, I.; Katerova, Z.; Shopova, E.; Dimitrova, L.; Brankova, L. Assessment of the Biochemical Responses of Wheat Seedlings to Soil Drought after Application of Selective Herbicide. *Plants* **2021**, *10*, 733. https://doi.org/10.3390/plants10040733

Academic Editor: Svetlana Misheva

Received: 18 March 2021
Accepted: 6 April 2021
Published: 9 April 2021

1. Introduction

During the last decades, human activity and the unwise use of natural resources has contributed to the continually deepening adverse climate changes on Earth. NASA reports [1] show that since the late twentieth century the average temperature has increased by 0.8 °C, which along with the increasing frequency of climatic anomalies such as periods of heavy drought or torrential rainfalls cause significant losses in the yield of important crops. Based on a number of climate models, it is predicted that in many regions the environmental changes will deepen and lead to even more tangible decline in plant productivity [2].

Drought is one of the environmental factors affecting almost all aspects of plant development. Physiological drought in plants can occur due to water scarcity or soil

(Thermo Electron Corporation, Vantaa, Finland). The enzyme activities were measured on Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). A refrigerated Sigma 2-16K centrifuge (SciQuip, Wem, UK) was also used in the experiments.

4.3. Statistics

The experiments were repeated three times. The samples were collected in three replicates each. The data presented in the Figures are mean values ± SE. The significance of the treatments was assessed by one-way ANOVA with post-hoc Duncan's multiple range test at $p < 0.05$.

5. Conclusions

The application of Serrate® did not alter considerably biochemical, phenotypic, and growth traits of wheat plants during the experimental period. Drought stress substantially inhibited plant growth and provoked an increase in the studied biochemical parameters. We found that the stress markers, enzymatic and non-enzymatic antioxidant defense were additionally increased during the stress period after the combined herbicide+drought treatment. The recovery of the herbicide + drought treated plants was comparable to the one witnessed in the individuals subjected only to drought. It could be concluded that Serrate® modulates the biochemical responses of wheat seedlings grown under drought stress but its action under adverse environment could not be explicitly characterized as cross-synergism or cross-adaptation without additional analyses.

Author Contributions: I.S. and D.T.—conceptualized and coordinated the research; L.D., Z.K., and D.T.—grown and treated the plants; I.S., D.T., E.S., L.B., L.D., and Z.K.—performed the laboratory analyses, collected, and interpreted the data; I.S.—prepared Figures; D.T. and Z.K.—prepared original draft of manuscript; I.S. and D.T.—reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Bulgarian National Scientific Fund (BNSF) grant KP-06-N36/3 (30.09.2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Biochemical Alterations in Triticale Seedlings Pretreated with Selective Herbicide and Subjected to Drought or Waterlogging Stress

Zornitsa Katerova , Dessislava Todorova, Elena Shopova , Liliana Brankova, Ljudmila Dimitrova, Margarita Petrakova and Iskren Sergiev *

Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria; zornitsa@bio21.bas.bg (Z.K.); dessita@bio21.bas.bg (D.T.); kostei@abv.bg (E.S.); lbrankova@abv.bg (L.B.); dim.lyudmila@gmail.com (L.D.); margarita.p02@abv.bg (M.P.)

* Correspondence: iskren@bio21.bas.bg

Abstract: Waterlogging and drought disrupt crop development and productivity. Triticale is known to be relatively tolerant to different stress factors. In natural conditions, plants are rather subjected to multiple environmental factors. Serrate® (Syngenta) is a systemic selective herbicide suitable for cereal crops such as triticale and wheat to restrain annual grass and broadleaf weeds. Triticale (×*Triticosecale* Wittm., cv. Rozhen) was grown as soil culture under controlled conditions. Seventeen-day-old plantlets were leaf sprayed with Serrate®. The water stress (drought or waterlogging) was applied after 72 h for 7 days, and then the seedlings were left for recovery. The herbicide does not provoke sharp alterations in the antioxidant state (stress markers level, and antioxidant and xenobiotic-detoxifying enzymes activity). The water stresses and combined treatments enhanced significantly the content of stress markers (malondialdehyde, proline, hydrogen peroxide), non-enzymatic (total phenolics and thiol groups-containing compounds), and enzymatic (activities of superoxide dismutase, catalase, guaiacol peroxidase, glutathione reductase) antioxidants, and xenobiotic-detoxifying enzymes (activities of glutathione S-transferase, NADPH:cytochrome P450 reductase, NADH:cytochrome *b5* reductase). These effects were more severely expressed after the drought stress, suggesting that this cultivar is more tolerant to waterlogging than to drought stress.

Keywords: antioxidants; Serrate®; stress markers; triticale; water stress; xenobiotic-detoxifying enzymes

Citation: Katerova, Z.; Todorova, D.; Shopova, E.; Brankova, L.; Dimitrova, L.; Petrakova, M.; Sergiev, I. Biochemical Alterations in Triticale Seedlings Pretreated with Selective Herbicide and Subjected to Drought or Waterlogging Stress. *Plants* **2023**, *12*, 2803. https://doi.org/10.3390/plants12152803

Academic Editor: Andrzej Bajguz

Received: 26 June 2023
Revised: 21 July 2023
Accepted: 27 July 2023
Published: 28 July 2023

1. Introduction

Triticale (×*Triticosecale* Wittm.) is a crop, artificially obtained after the hybridization of wheat (*Triticum* spp.) with rye (*Secale cereale* L.), which became commercially available approximately a half century ago [1,2]. It is assumed to be relatively tolerant to environmental challenges, due to its rye traits, and it has high genetic diversity for abiotic stress responses [2–4]. Drought and waterlogging are among the devastating abiotic stresses, which influence negatively crop yield [5]. Water stresses (drought and waterlogging) negatively affect photosynthesis, lead to redox imbalance and oxidative stress, alter optimal physiological and biochemical processes and ultimately reduce crop yield [6–8]. These stresses were studied in detail when applied individually in both field and laboratory conditions [9]. In natural conditions, plants are subjected to combinations of different factors, but the information for the effects is insufficient [10,11]. There are few articles reporting the effect of abiotic stress combination (including herbicides) on the biochemical status of crops [12–17]. Generally, the resultant effect of multiple treatments is specific and unpredictable [9,18,19]. The information if the effect of treatment combinations on plants is negative (cross-synergism) or positive (cross-adaptation) could be obtained experimentally.

Among other specific physiological alterations, environmental stresses force plants to intensify accumulation of different reactive oxygen species (ROS) [6,20,21]. Depend-the inhibition of the photochemical reduction of nitroblue tetrazolium was used. The amount of the enzyme required to inflict a 50% inhibition was defined as one unit of SOD [70]. The activity of glutathione reductase was assessed by following the method described by Smith et al. [71]. The reaction mixture contained 100 µL supernatant, 1.180 mL reaction buffer (0.05M potassium phosphate buffer, pH 7.5, 1 mM EDTA), 20 µL 50 mM DTNB, 0.1 mL 7.5 mM oxidized glutathione and 0.1 mL 1.5 mM NADPH. The reaction was assessed at 412 nm for 60 s. The GST activity with 1-chloro-2,4-dinitrobenzene (CDNB, extinction coefficient 9.6 mM cm^{-1} at 340 nm) as a substrate was determined according to Gronwald et al. [72].

Cold 100 mM potassium phosphate buffer (pH 7.5), containing 1 mM EDTA, 0.2 mM PMSF, 1% PVP and 0.3 M sucrose, was used as an extraction solution during the homogenization of the plant material for the assessment of the enzymatic activities of B5R and CPRs. The homogenate was centrifuged at 15,000× *g* for 30 min at 4 °C. Potassium ferricyanide was used as an artificial electron acceptor for the in vitro B5R (EC 1.6.2.2) activity assay [73]. The rate of reduction was evaluated by an extinction coefficient of 1.02 mM cm^{-1} at 420 nm. Cytochrome *c* was used as an artificial electron acceptor for the in vitro CPR (EC 1.6.2.4) activity assay [73]. The rate of reduction was evaluated by an extinction coefficient of 21.1 mM cm^{-1} at 550 nm. The protein content was determined according to Bradford [74]. The herbicide Serrate® was purchased from a local distributor of Syngenta (Basel, Switzerland). All chemical compounds used for the biochemical analyses were obtained from Sigma-Aldrich, (Saint Louis, MO, USA). The measurements of the stress markers, SOD activity and non-enzymatic antioxidants were performed on a Multiskan Spectrum spectrophotometer with a microplate reader (Thermo Electron Corporation, Vantaa, Finland). The enzymatic activities were measured on a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). A refrigerated Sigma 2-16K centrifuge (SciQuip, Wem, UK) was used to obtain the respective supernatants.

4.3. Statistical Analysis

The results presented are obtained from three independent biological experiments with three internal replicates. The statistical significance between the treatments were evaluated using one-way ANOVA with a post-hoc Duncan's multiple range test ($p \leq 0.05$). The data in the figures represent the average values ± standard error (SE).

Author Contributions: I.S. and D.T., conceptualized and coordinated the research; L.D., Z.K., M.P. and D.T., grew and treated the plants; I.S., D.T., E.S., L.B., M.P. and Z.K., performed the laboratory analyses, collected and interpreted the data; I.S., prepared figures and photos; D.T. and Z.K., prepared original draft of manuscript; I.S. and D.T., reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the grant KP-06-N36/3 (30.09.2020) of National Science Fund, Ministry of Education and Science, Republic of Bulgaria.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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

Tome 75, No 7, 2022

AGRICULTURAL SCIENCES

Plant breeding

MICROSOMAL P450-RELATED ELECTRON TRANSFER COMPONENTS, GLUTATHIONE AND GLUTATHIONE S-TRANSFERASE CONTRIBUTION IN STRESS RESPONSE OF HERBICIDE-TREATED WHEAT TO DROUGHT AND WATERLOGGING

Liliana Brankova, Ljudmila Dimitrova, Elena Shopova, Zornitsa Katerova[#], Iskren Sergiev, Dessislava Todorova

Received on January 20, 2022
Presented by A. Atanassov, Member of BAS, on February 24, 2022

Abstract

The activities of some plant microsomal P450-related electron transfer components (NADPH-cytochrome P450 reductase, CPR and NADH-cytochrome *b5* reductase, B5R), glutathione S-transferase (GST) together with the content of reduced (GSH) and oxidized (GSSG) glutathione were determined in wheat (*Triticum aestivum* L., cv. Sadovo 1) treated with herbicide Serrate® prior to drought or waterlogging. Both stresses, especially drought, increased the content of glutathione and the activities of CPR and B5R during the stress, while GST activity was rather decreased. Herbicide application alone increased substantially most of the parameters, especially at the beginning of the experimental period. Serrate®+drought/waterlogging (S+D/W), amplified the observed alterations (except for GST), which were more significant after drought. The decrease in ratio GSH to GSSG after 4 days of recovery for all treatment groups except Serrate® signifies disrupted buffer capacity in cells. After 4 days of recovery the activities of CPR and B5R dropped below the control, while the glutathione pool and GST activity remained increased in drought and S+D

[#]Corresponding author.

This work is supported by the National Science Fund, Ministry of Education and Science, Republic of Bulgaria (Grant KP-06-N36/3-30.09.2020).

DOI:10.7546/CRABS.2022.07.18

treated plants. Contrariwise, the levels of glutathione, CPR and B5R remained closer to control in waterlogged plants, while S+W kept higher levels of P450-related enzymes. The herbicide application prior to drought or waterlogging did not affect negatively detoxification and oxidative status of wheat plants compared to those subjected to water stress alone.

Key words: water stress, selective herbicide, herbicide detoxification

Abbreviations: CPR – NADPH-cytochrome P450 reductase, B5R – NADH-cytochrome *b5* reductase, GST – glutathione S-transferase, GSH – reduced glutathione, GSSG – oxidized glutathione, ROS – reactive oxygen species, S+D – Serrate®+drought, S+W –Serrate®+waterlogging

Introduction. Application of herbicides is an ordinary practice in agriculture to restrain weed growth. Serrate® (Syngenta) is a selective herbicide used in wheat cultivation. It contains two active compounds (clodinafop-propargyl and pyroxsulam) with different modes of action and inhibits the biosynthesis of fatty acids and amino acids, respectively. Serrate® is commonly used to cope with the annual grass and broadleaf weeds in cereal crops, especially in wheat, because of its tolerance. Drought and waterlogging are common worldwide abiotic stresses affecting negatively various aspects of plant growth and development and decreasing plant production. Oxidative stress is a common stress response leading to overproduction of reactive oxygen species (ROS) which may possess signalling or destructive functions depending on ROS type, cellular sites of production, concentration, etc. [1,2]. Depending on the balance between the production of ROS and their scavenging by the antioxidative defense systems plants may adapt or not. Antioxidative defense system consists of non-enzymatic (glutathione, ascorbate, phenolics, etc.) and enzymatic (superoxide dismutase, catalase, peroxidase, glutathione S-transferase, etc.) components acting together to sustain the homeostasis. NADPH:cytochrome P450 reductase (CPR) is a membrane-bound flavoprotein, localized primarily in the endoplasmic reticulum membrane. Its function is to transfer reducing equivalents from NADPH to diverse P450 monooxygenases, involved in various secondary metabolic reactions, including biosynthesis of phenylpropanoids, terpenoids, alkaloids, fatty acids, plant hormones, etc. [3–6]. NADH:cytochrome b_5 reductase (B5R) is localized in the endoplasmic reticulum, where it transfers electrons from NADH to cytochrome b_5 and then to various lipid-modification reactions, like fatty-acid and sterol precursors desaturation, and fatty acids hydroxylation as well as P450 mediated reactions [7,8]. Both enzymes are involved in plant defense mechanisms against diverse abiotic stresses [4,6]. The CPR and B5R, as well as glutathione and GST participate in Phase I and Phase II of xenobiotic catabolism, and herbicides detoxification in particular [3]. These defense compounds take part in antioxidative defense shield toward various stresses [4–6,9].

Recently multifactorial stress combinations have become more frequent within arable land but they are still scarcely studied in plants [10]. To our knowledge

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

Tome 75, No 6, 2022

AGRICULTURAL SCIENCES

Plant breeding

INVOLVEMENT OF POLYAMINES IN PHYSIOLOGICAL REACTIONS OF HERBICIDE-TREATED WHEAT SEEDLINGS SUBJECTED TO DROUGHT AND WATERLOGGING STRESS

Dessislava Todorova, Zornitsa Katerova, Ljudmila Dimitrova, Iskren Sergiev#

Received on December 14, 2021
Presented by A. Atanassov, Member of BAS, on January 27, 2022

Abstract

Polyamines are plant growth regulators, which take part in plant growth and development, as well as in the physiological responses to diverse biotic and abiotic stresses. Drought and waterlogging are environmental stress factors that disturb normal plant growth. In our study, we determined the content of polyamines spermine, spermidine, and putrescine in young wheat seedlings (*Triticum aestivum* L., cv. Sadovo-1) pretreated with herbicide Serrate® (Syngenta) and subjected for 7 days to drought or waterlogging. We found that when applied alone the herbicide caused some decrease in polyamine levels but it was not substantial as compared to drought and waterlogging stresses. Obvious reduction of polyamine content was caused by both stress factors when applied alone or in combination with the herbicide. The decrease was more significant in drought-stressed seedlings than in waterlogged. When plants were transferred to normal irrigation regime the polyamine concentrations in drought-stressed plants tended to increase. The waterlogging stress continued to reduce polyamine content even during the recovery period. These data correlate with the growth parameters (fresh weight, height of shoots) indicating the

#Corresponding author.

This work was supported by National Science Fund, Ministry of Education and Science, Republic of Bulgaria (Grant KP-06-N36/3-30.09.2020).

DOI:10.7546/CRABS.2022.06.17

involvement of polyamines in the physiological responses of herbicide-primed wheat seedlings under stress conditions.

Key words: drought, putrescine, spermidine, spermine, *Triticum aestivum* L., waterlogging

Abbreviations: PAs – polyamines, Put – putrescine, ROS – reactive oxygen species, Spd – spermidine, Spm – spermine, TCA – trichloroacetic acid

Introduction. Polyamines (PAs) putrescine (Put), spermidine (Spd), and spermine (Spm) are organic low-molecular-weight nitrogen-containing compounds, which contribute to a number of important growth and developmental processes in plants. They also have free radical scavenging properties and antioxidant activity, and participate in the plants physiological responses to diverse biotic and abiotic stress factors [1]. PAs can be found in plants as free molecules, but due to their polycationic nature they also can conjugate with small molecules (phenolic acids) or with macromolecules like proteins and nucleic acids [2].

Drought limits the growth of many crops more than any other environmental factors and considerably decreases production's quality and quantity. Water shortage provokes a reduction in growth rate, stem elongation, leaf expansion and stomata movements, causes changes in principal physiological and biochemical processes and thus reflects the plant growth and productivity. Waterlogging is another substantive impediment to agriculture. It can be a result of unforeseen and intensive rainfalls leading to bad soil drainage and flooding. Water excess negatively influences plant growth, development, and crop production yield due to disturbance in the root hydraulic conductivity, limitation of oxygen access and CO_2 assimilation, decrease in photosynthesis and respiration [3].

Nowadays, usage of herbicides to control weeds is a necessary part of the contemporary agriculture. Serrate® (Syngenta) is a systemic and selective herbicide for wheat, rye and triticale. It controls annual grass and broadleaf weeds due to its dual composition of active ingredients – clodinafop-propargyl (inhibitor of fatty acids biosynthesis) and pyroxsulam (inhibitor of branched-chain amino acids biosynthesis). In nature, plants are usually exposed to more than one unfavourable factors. Recently, we reported data on the enzymatic and non-enzymatic antioxidant response of wheat plants treated with Serrate® herbicide and subsequently exposed to drought or waterlogging stress [4,5]. In the present study we aimed to investigate the combined effects of herbicide and subsequent drought or waterlogging stress on the polyamine pool in Bulgarian wheat cultivar Sadovo-1. The alterations in polyamines content due to Serrate® applied alone or in combination with unfavourable growth conditions have not been studied up to now.

Material and methods. ***Plant material and treatments.*** Seventeen-day-old soil-grown wheat seedlings (Triticum aestivum L., cv. Sadovo-1) were foliar sprayed with water solution containing Serrate® herbicide and Adigor® (adjuvant) according to the manufacturer instructions. Pretreated plantlets were

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

Tome 77, No 1, 2024

AGRICULTURAL SCIENCES

Plant breeding

POLYAMINE ALTERATIONS OF TRITICALE IN RESPONSE TO HERBICIDE, DROUGHT AND WATERLOGGING TREATMENTS

Zornitsa Katerova, Asya Petrova, Iskren Sergiev, Dessislava Todorova✉

Received on September 4, 2023
Presented by H. Najdenski, Corresponding Member of BAS, on November 28, 2023

Abstract

Drought and waterlogging are environmental stress factors that havoc normal plant growth and development. Polyamines are plant growth regulators, which participate in plant growth and development, as well as in the physiological responses to stress conditions. The polyamines fractions were evaluated in triticale plants pretreated with the selective herbicide Serrate®, and exposed to drought or waterlogging for 7 days. The herbicide applied alone provoked a slight decrease in polyamine content. A decrease of polyamine content was found also after drought, while waterlogging provoked an increase in polyamine levels. These data correlate with plant fitness and phenotype characteristics of treated seedlings. Our data provide new information about the role of plant polyamines in the physiological responses of triticale plants to water stress after herbicide application.

Key words: putrescine, spermidine, spermine, ×*Triticosecale* Wittm., water stress

Abbreviations: PAs – polyamines, Put – putrescine, ROS – reactive oxygen species, Spd – spermidine, Spm – spermine, TCA – trichloroacetic acid

Introduction. Putrescine (Put), spermidine (Spd), and spermine (Spm) are the most abundant polyamines (PAs) in plants. They are among the most extensively studied plant growth regulators. PAs might be detected in free form

This work is supported by National Science Fund, Ministry of Education and Science, Republic of Bulgaria (Grant KP-06-N36/3-30.09.2020).

https://doi.org/10.7546/CRABS.2024.01.18

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

Tome 58, No 3, 2005

BIOLOGIE
Physiologie des plantes

EFFECTS OF LONG-TERM TREATMENT WITH LOW CONCENTRATIONS OF HERBICIDES ATRAZINE, GLYPHOSATE AND 2,4D ON IAA OXIDASE ACTIVITY IN YOUNG PEA PLANTS

S. Ivanov, Z. Katerova, E. Ivanova, V. Alexieva

(*Submitted by Academician E. Karanov on November 24, 2004*)

Abstract

Endogenous IAA content in plant tissues can be modulated via oxidative decarboxylation on the side chain of IAA by IAA oxidase. In plants IAA oxidase activity displays some isoforms of unspecific peroxidases. Usually, stimulation in IAA oxidase activity after treatment with various stress agents corresponded with reduction of endogenous IAA content and growth inhibition. Atrazine, 2,4D and glyphosate have been widely used as herbicides in crop production. The aim of this study was to evaluate the effects of long-term treatment with low concentrations of these herbicides on IAA oxidase activity in young pea plants. Plants were grown hydroponically. Atrazine and 2,4D were added to the nutrition medium in concentration 0.1 μM and 1 μM, and glyphosate in 1 μM and 10 μM, respectively. Leaf material was collected 7 and 14 days after the beginning of the experiment. In general, long-term influence with low concentrations of 2,4D and glyphosate enhanced, and atrazine did not provoke significant changes in IAA oxidase activity. On the basis of our results and previous research in this area we concluded that the changes in IAA oxidase activity strongly correlate with total peroxidase activity in plant cell. Additionally, we speculated that the reduction of IAA content by IAA oxidase activity could be a secondary effect from the activation of some unspecific peroxidases.

Key words: auxin, IAA oxidase, peroxidase, atrazine, 2,4D, glyphosate, pea

Abbreviations: AOS – reactive oxygen species; IAA – indole-3-acetic acid; 2,4D – 2,4-dichlorophenoxyacetic acid

Introduction. Auxins affect numerous processes during plant growth and development including cell division and elongation, differentiation, apical dominance, tropisms, senescence, and flowering. Endogenous IAA content in plant tissues can be modulated via several pathways including synthesis, decomposition and transport of free or conjugated IAA. One well knows that IAA metabolic pathway consists of aerobic oxidative decarboxylation on the side chain of IAA by IAA oxidase leading to formation of either indole-3-methanol or 3-methylene oxindole [1]. It is known since 1955 [2] that IAA oxidation is catalyzed by horseradish peroxidase. In tobacco cell lines BY-2 IAA oxidase activity exhibits only P-type basic guaiacol peroxidase [3]. Subcellular localization of IAA oxidase in etiolated pea epicotyls was studied by Waldrumi and Davies [4]. Their results demonstrated that the enzyme activity is associated most closely with Golgi apparatus and to a lesser degree with lysosomes and endoplasmatic reticulum. In most of the research works in this area the rise of IAA oxidase activity

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Article

Modulation of Physiological Stress Response of *Triticum aestivum* L. to Glyphosate by Brassinosteroid Application

Elena Shopova [1], Zornitsa Katerova [1,*], Liliana Brankova [1], Ljudmila Dimitrova [1], Iskren Sergiev [1], Dessislava Todorova [1] and Neveen B. Talaat [2]

1 Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; kostei@abv.bg (E.S.); lbrankova@abv.bg (L.B.); dim.lyudmila@gmail.com (L.D.); iskren@bio21.bas.bg (I.S.); dessita@bio21.bas.bg (D.T.)

2 Department of Plant Physiology, Faculty of Agriculture, Cairo University, Giza 12613, Egypt; neveenbt@yahoo.com

* Correspondence: zornitsa@bio21.bas.bg

Abstract: The potential of brassinosteroids to modulate the physiological responses of winter wheat (*Triticum aestivum* L.) to herbicide stress was evaluated. Young winter wheat seedlings were treated with 24-epibrassinolide (EBL) and 24 h later were sprayed with glyphosate. The physiological responses of treated plants were assessed 14 days after herbicide application. Wheat growth was noticeably inhibited by glyphosate. The herbicide application significantly increased the content of the stress markers proline and malondialdehyde (MDA) evidencing oxidative damage. The content of phenolic compounds was decreased in the herbicide-treated plants. Slight activation of superoxide dismutase (SOD) and catalase (CAT) and considerable increase of glutathione reductase (GR) and guaiacol peroxidase (POX) activities were found. Increased POX and glutathione S-transferase (GST) activities were anticipated to be involved in herbicide detoxification. Conjugation with glutathione in herbicide-treated plants could explain the reduction of thiols suggesting unbalanced redox state. EBL application did not alter the plant growth but a moderate activation of antioxidant defense (POX, GR, and CAT activities and phenolic levels) and detoxifying enzyme GST was observed. The hormonal priming provoked a slight decrease in MDA and proline levels. The results demonstrate that EBL-pretreatment partly restored shoot growth and has a potential to mitigate the oxidative damages in glyphosate-treated plants through activation of the enzymatic antioxidant defense and increase of the phenolic compounds.

Keywords: wheat; herbicide; brassinosteroids; abiotic stress

Citation: Shopova, E.; Katerova, Z.; Brankova, L.; Dimitrova, L.; Sergiev, I.; Todorova, D.; Talaat, N.B. Modulation of Physiological Stress Response of *Triticum aestivum* L. to Glyphosate by Brassinosteroid Application. *Life* **2021**, *11*, 1156. https://doi.org/10.3390/life11111156

Academic Editor: Balazs Barna

Received: 21 September 2021
Accepted: 27 October 2021
Published: 29 October 2021

1. Introduction

Herbicides are often used in modern agricultural practice to manage weed growth. Glyphosate (N-(phosphonomethyl)glycine) use has increased 15-fold from 1994 to 2014 on a worldwide scale [1]. It is the most extensively used herbicide, consequently, weed resistance to its usage has emerged. As a result, non-target contamination occurred bringing negative outcomes for a number of native plant species [2–8]. The introduction of glyphosate-resistant crops in agricultural practice caused an additional rise in the use of the herbicide in soybean, corn, cotton, and wheat cultivation [3,9]. Glyphosate is a broad-spectrum, non-selective herbicide, killing all affected plants. It inhibits the plastidic 5-enolpyruvylshikimate-3 phosphate synthase, a key enzyme in the shikimate pathway. Consequently the biosynthesis of aromatic amino acids and their essential derivatives (hormones, enzyme cofactors, etc.) is blocked [2,3,10]. The glyphosate principal metabolite, aminomethylphosphonic acid, which is admitted to be a phytotoxin, may occur in some plants and enhance the negative effects of the herbicide [10,11]. Due to the cation chelator features of glyphosate, it may block important enzymatic cofactors and affect enzymatic antioxidants and photosynthesis [10,12]. The boost of reactive oxygen species (ROS) in activity by glyphosate treatment and this effect was strengthened after the EBL + glyphosate application. Similar alterations in the content of H_2O_2, and the activities of POX and CAT were reported earlier in *Brassica juncea* L. or rice plantlets [34,47] after EBL seed-priming. The increased activity of POX could be an important element of xenobiotics metabolism [32,61]. In general, peroxidases are able to take part in both H_2O_2 augmentation or utilization trough oxidative cycle or peroxidative cycle, respectively [62,63]. In the classical peroxidative cycle H_2O_2 is used to transform phenolic substrate compounds into corresponding phenoxy radicals which can consequently make final products as lignins [62,63]. In the oxidative cycle NADH is oxidized as electron donor to $NAD^{\bullet}$ radical, which subsequently reduces the oxygen to superoxide radical [63]. Subsequently the "NADH-oxidase" activity of peroxidase results in H_2O_2 synthesis with or without the contribution of SOD [62]. However, the slight induction of SOD after herbicide treatment in our study indicates the necessity the superoxide radical to be scavenged in these plants.

EBL pretreated plants were previously reported to increase *GST* expression or GST activity in combination with other pesticides or xenobiotics [32,34]. The presented results show that GST was involved in the detoxification process in the EBL pretreated experimental group. The stunted growth and increased lipid peroxidation caused by glyphosate was partially alleviated by the EBL pretreatment. This was accompanied by increased total phenolics content and higher activities of POX, CAT, GST and GR.

5. Conclusions

We confirmed that glyphosate treatment reduces substantially wheat growth and inhibits the non−enzymatic defense (thiols and phenolic compounds) of the affected plants. The herbicide negative influence on the physiological status of the plants is manifested by the increased amounts of the stress markers proline and MDA and the activation of the antioxidant and the xenobiotic detoxification enzymes. Applied alone EBL slightly reduced stress markers (MDA and proline), enhanced phenolic compounds and induced enzymatic defense system (POX, GR, GST and CAT). We demonstrate that EBL pretreatment improves the performance of wheat plants subjected to glyphosate as evidenced by the partially restored shoot growth and the reduction of the lipid peroxidation marker MDA. Our data support earlier findings that brassinosteroids promote the metabolism of pesticides and are crucial for their proper detoxification [32,33,61]. It could be concluded that the exogenous EBL induces xenobiotic detoxification through activation of POX and GST. As a result the activation of the antioxidant defense probably exerts a protective role towards the negative effects of the glyphosate action.

Author Contributions: D.T. and N.B.T.—conceptualized and coordinated the research; L.D., Z.K. and D.T.—responsible for the plant cultivation and treatment procedures; I.S., D.T., E.S., L.B., L.D. and Z.K.—performed the laboratory analyses, collected and interpreted the data; Z.K.—prepared the figures; Z.K.—prepared the original draft of the manuscript; I.S., Z.K. and D.T.—reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: This study was supported by Bulgaria-Egypt joint research program of Bulgarian Academy of Sciences (2019–2021).

Conflicts of Interest: The authors declare no conflict of interest.

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CHAPTER 35

Polyamines and brassinosteroids in drought stress responses and tolerance in plants

Dessislava Todorova[1], Neveen B. Talaat[2], Zornitsa Katerova[1], Vera Alexieva[1], and Bahaa T. Shawky[3]

[1] *Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[2] *Department of Plant Physiology, Faculty of Agriculture, Cairo University, Giza, Egypt*

[3] *Department of Microbial Chemistry, Genetic Engineering and Biotechnology Research Division, National Research Center, Giza, Egypt*

35.1 Introduction

Drought limits the growth of many crop varieties more than any other environmental factor and decreases the production quality and quantity. Water shortage provokes a reduction in growth rate, stem elongation, leaf expansion and stomata movements, causes changes in principal physiological and biochemical processes and thus reflects on plant growth and productivity (Du *et al.*, 2010; Ambrosone *et al.*, 2011; Boughalleb and Hajlaoui, 2011; Filek *et al.*, 2012; Hosseini Boldaji *et al.*, 2012; Vaseva *et al.*, 2012; Badran *et al.*, 2015; Griesser *et al.*, 2015; Mutava *et al.*, 2015; Paul *et al.*, 2015; Yin *et al.*, 2015). Plants subjected to water stress undergo an increased exposure to the reactive forms of oxygen (ROS), and the accumulation of free radicals provokes damage to biomembranes (Gill and Tuteja, 2010b). During their phylogenesis plant organisms have developed protective systems to cope with the deleterious effects of unfavorable environmental conditions, which include a complex of non-enzymatic and enzymatic antioxidants (Gill and Tuteja, 2010b; Talbi *et al.*, 2015). Additionally, under water stress, plants accumulate significant amounts of compatible solutes such as free amino acids, soluble sugars, proline, and glycinebetaine. Plant organisms are capable of overcoming oxidative stress by activation of some or all of these systems. However, in some cases, the capacity of defense systems is not enough to overcome the destructive effects of ROS, which leads to considerable damage and even to plant death. Endogenous plant defense systems could be strengthened by application of plant growth substances. There is much evidence that different plant growth regulators (PGRs) can modulate plant responses to unfavourable environmental factors (Todorova *et al.*, 2008; Vardhini *et al.*, 2011a,b). It was proposed that exogenous application of some PGRs should decrease membrane damage, enhance plant defence systems against harmful ROS effects, and increase organic solute accumulation.

Polyamines are organic nitrogen-containing compounds with a low molecular weight and C_3-C_{15} aliphatic structure. Polyamines have at least two primary amino groups and one or more internal imino groups. The triamine spermidine (Spd), tetraamine spermine (Spm), and their precursor the diamine putrescine (Put), are the major polyamines (PAs) that are spread among all plant species (Figure 35.1). Polyamines contribute to a number of important growth and developmental processes in plants like regulation of gene expression; cell division, growth, and differentiation; dormancy breaking of tubers and germination of seeds; embryo-, organo-, and morphogenesis; stimulation, support, and development of flower buds; fruit set, growth, and ripening; and cell, organ, and tissue senescence. Due to their polycationic nature, polyamines possess antioxidant properties and free radical scavenging activity and this contributes to plant tolerance to different abiotic stresses, including drought (Groppa and Benavides, 2008; Gill and Tuteja, 2010a; Todorova *et al.*, 2014). Brassinosteroids (Figure 35.2) are a class of plant steroid hormone that regulate multiple physiological

Water Stress and Crop Plants: A Sustainable Approach, Volume 2, First Edition. Edited by Parvaiz Ahmad.