BIOLOGIJA. 2007. Vol. 53. No. 2. P. 67–70

The UV-B impact upon the enzyme of antioxidant system superoxide dismutase (SOD) of potato somatic hybrids

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Plant response to the UV-B stress and evaluation of the role of the antioxidant enzyme SOD (SOD EC 1.15.1.1) in adaptation mechanisms of frost-resistant somatic hybrids of *Solanum tuberosum* and *Solanum commersonii* as well as their paternal forms have been investigated. After UV-B impact, leaf area, dry weight and the concentration of chlorophyll a and b decreased. However, antioxidant activity of SOD increased in all test plants, but the frost-resistant potato hybrids demonstrated a higher SOD level than the frost-sensitive *S. tuberosum* 'Matilda', and even a higher level of tolerance towards the other abiotic factor, UV-B irradiation, was observed. An increased activity of SOD after UV-B irradiation should be regarded as the adaptive response of a plant to oxidative stress common in both frost treatment and UV-B irradiation. It confirms the participation of SOD isoforms in the adaptation and protection mechanisms.

Key words: frost resistance, UV-B response, superoxide dismutase (SOD EC 1.15.1.1), *Solanum tuberosum, S. commersonii*, frost-resistant somatic hybrids

INTRODUCTION

Cultivated plants are usually more sensitive to abiotic stress. Wild plant species are better adapted to the changing environmental conditions, such as drought, cold, salinity, increased UV-B irradiation [1]. Under usual growth conditions, the reactive oxygen species (ROS) in cells are regulated in the cell metabolism. However, ROS triggered by any of these environmental stresses can cause changes in subcellular components as well as in the plant genome. It is rather usual that plants adapted to grow under certain conditions tolerate well the impact of another stress [2]. The mechanisms of detoxication are involved to prevent the plants from damage. One of them is the enzyme systems. Enzymatic defense mechanisms include different enzymes – superoxide dismutase, catalase and peroxidase. Numerous references report a correlation between the increased resistance to environmental stress with and SOD activity [1, 3, 4]. The aim of this study was to investigate the response of the SOD in frost-resistant potato hybrids to oxidative stress caused by UV-B. Frost is one of the environmental stress factors that damage potato plants. The influence of UV-B on the oxidative stress enzyme superoxide dismutase (SOD) of frost-resistant somatic potato hybrids was studied. Frost resistance was incorporated by protoplast fusion from the wild species *S. commersonii* into the frost-sensitive *S. tuberosum* 'Matilda' and 'Venta' [5]. The antioxidant enzyme systems may be involved in the plant defense mechanisms against oxidation damage.

MATERIALS AND METHODS

Plant material and growth conditions. The plants were cultivated in soil pots. One potato tuber per pot was grown. In the chambers, OSRAM L 36/77 Fluora (PAR 53 μm m^{-2} s^{-1}) lamps were used for illumination. The temperature was above 25 °C with 16/8 h light and darkness photoperiods. Two separate experiments with UV-B irradiation were performed. Before UV-B irradiation, the plants had been grown for 2 (the first experiment, A) and 3 (the second experiment, B) weeks. In the first experiment (A), plants were two weeks old: the frost-sensitive *Solanum tuberosum* 'Matilda', frost-resistant wild species of *S. commersonii* and their frost-resistant somatic hybrids H 188, H 269, H 323, H 515. In the second experiment (B), plants were 3 weeks old: the frost-sensitive *Solanum tuberosum* 'Matilda', 'Venta', frost-resistant wild species *S. commersonii* as well as their frost-resistant somatic hybrids H 554, H 90, H 323, H 487.

UV-B irradiation conditions. Plants of experiments A and B were irradiated with TL 40 W/12 RS UV-B lamps (Philips). The UV-B doses were 8 kJ/m^2 a day. The irradiation lasted 8 days. UV-B-untreated plants served as a control. The plants were examined the next day after UV-B irradiation. Both experiments were made in three replications.

Plant growth measurements. The height of plants and their leaf area were measured. For leaf area, the four apical leaves were scanned and analysed with Sigma Scan Pro. The same leaves were used for the determination of fresh and dry leaf weight.

The concentration of chlorophyll a and b. Pigments were extracted with DMF (*N,N*–dimethylformamide) [6]. The concentration of chlorophyll a and b was determined with a spectrophotometer at 664 and 647 nm wavelengths.

Extraction of soluble proteins and SOD activity assay. Quantification of soluble proteins was performed according to Bradford [7] with bovine serum albumin as the standard. The total SOD activity of leaf extracts was assayed by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium and measured with a spectrophotemeter at 560 nm wavelength [8]. SOD isoforms in plant leaves were separated on native 9% PAG according to Laemmli [9].

Statistical analysis. The experiments were performed in three replicate. The statistical data were analysed employing the MS Excel 2002 package. The treatment effects were considered at $p = 0.05$.

RESULTS AND DISCUSSION

UV-B irradiation affected mostly morphological traits and reduced significantly the height and fresh weight of plants, thus diminishing their productivity. UV-B irradiation caused unequal response in different plant species and even in cultivars of the same species to oxidative stress [10, 11]. Comparison of the frost-resistant potato hybrids with the control showed a significant reduction in the height of all plants in both experiments; the reduction particularly evident was (data not shown) in the height of 2-week-old plants in the first experiment.

Our results showed a decrease of leaf area, fresh and dry weight of leaves after UV-B irradiation in the frost-resistant hybrids H 323, H 269 and H 188 (Figs. 1, 2). However, the leaf area of hybrid H 515 increased even more than in the control plants. Changes of the growth parameters were much more significant (Fig. 2 A) when 2-week-old plants had been irradiated, in comparison with the 3-week-old plants (Fig. 2 B). It is worth noting that younger plants were more sensitive to UV-B irradiation.

The amount of soluble proteins after UV-B irradiation slightly increased in 2-week-old hybrids (Fig. 3 A), but in 3-week-old hybrids the content of soluble proteins decreased, except for *S. tuberosum* cv. 'Venta' and hybrid H 487 (Fig. 3 B). A comparison of both experiments showed that the content of soluble proteins in *S. tuberosum* 'Matilda' and the wild species of *S. commersonii* did not alter significantly and was close to the control level after UV-B irradiation in both.

After UV-B exposure, the concentrations of chlorophyll a and b decreased in all hybrids as well as in paternal forms in both experiments.

The suppressed plant growth and reduced biomass production caused by oxidative stress are attributed to a decrease of the photosynthetic activity in plants [12].

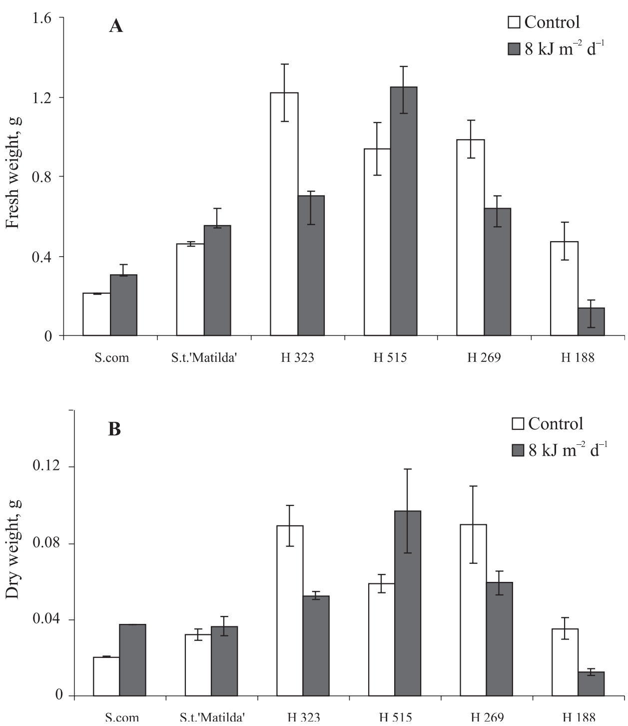

Fig. 1. Fresh (A) and dry (B) weight of 2-week-old frost-resistant somatic *S. commersonii (S. com.)* and *S. tuberosum (S. t.)* hybrids after 8 days of irradiation with UV-B 8 kJ m^{-2} d^{-1} dose: frost resistant wild species *S. commersoni,* frost-sensitive *S. tuberosum* 'Matilda' (*S. t.*'Matilda') and frost-resistant somatic hybrids H323, H 515, H 269, H 188

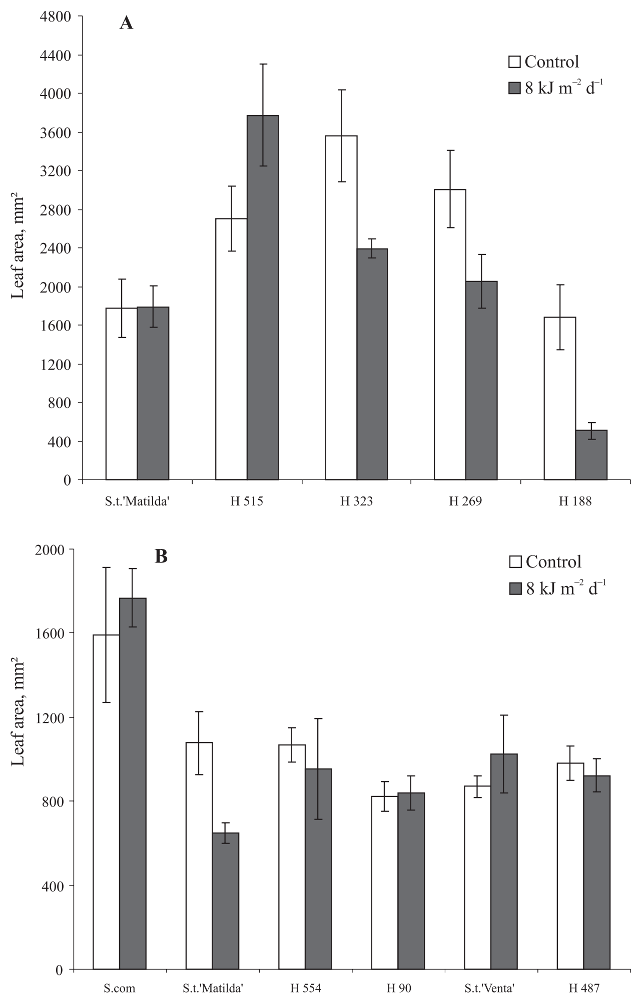

Fig. 2. Leaf area of frost-resistant somatic *S. commersonii* and *S. tuberosum* hybrids 2 weeks (A) and 3 weeks old (B) after 8 days of irradiation with UV-B 8 kJ m^{-2} d^{-1} dose. A: frost-resistant wild species *S. commersoni,* frost-sensitive *S. tuberosum* 'Matilda' and frost-resistant hybrids. Abbreviations as in Fig. 1. B: frost-resistant wild species *S. commersoni,* frost-sensitive *S. tuberosum* 'Matilda' and frost-resistant hybrids H 554, H 90, frost-sensitive *S. tuberosum* 'Venta' (*S. t.*'Venta') and the frost-resistant hybrid H 487

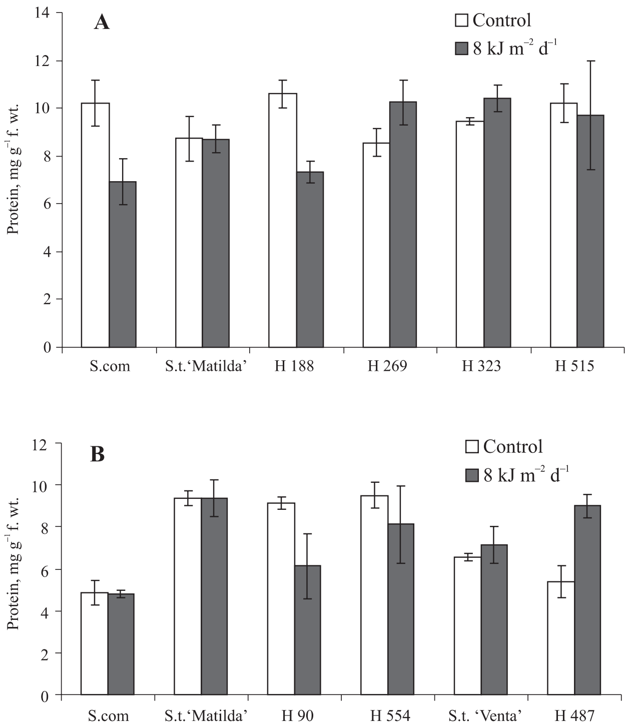

Fig. 3. Protein content in leaves of frost-resistant somatic hybrids of the *S. commersonii* and *S. tuberosum.* A – 2-week-old plants and B – 3-week-old plants after 8 days of irradiation with UV-B 8 kJ m^{-2} d^{-1} dose. Abbrevations as in Fig. 2

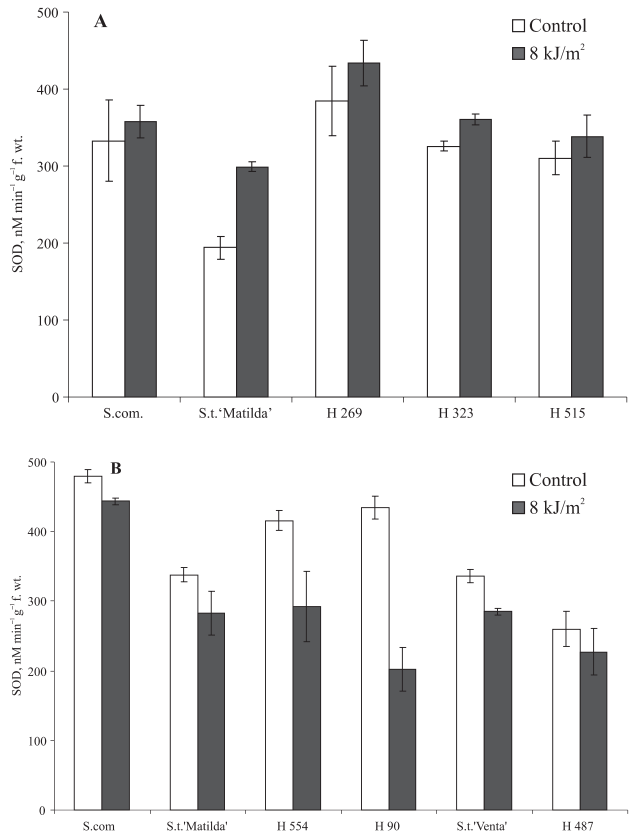

Fig. 4. Superoxide dismutase (SOD) activity in leaves of frost-resistant somatic of the *S. commersonii* and *S. tuberosum* hybrids 2 weeks (A) and 3 weeks old (B) after 8 days of irradiation with UV-B 8 kJ m^{-2} d^{-1}. Abbrevations as in Fig. 2

Comparison of frost-sensitive *S. tuberosum* 'Matilda' and frost-resistant *S. commersonii* after UV-B impact revealed the significance of the antioxidative enzyme systems. The SOD activity in control plants of the frost-resistant species *S. commersonii* was 1.7 times higher than that of the frost-sensitive *S. tuberosum* 'Matilda' (Fig. 4 A). The UV-B irradiation increased SOD activity in *S. tuberosum* 'Matilda' 1.14 times, and SOD activity reached the level of the wild species *S. commersonii*. SOD activity in the frost-resistant hybrids H 269 and H 323 increased 1.1–1.3 times under the impact of UV-B irradiation versus the frost-resistant wild species *S. commersonii*. It is obvious that SOD activity in the UV-B-irradiated plants increased in response to UV-B stress. The frost-resistant potato hybrids demonstrated even a higher SOD activity than did *S. commersonii* in which SOD activity was about twice as high as in *S. tuberosum* leaves (Fig. 4 A).

However, UV-B irradiation of 3-week-old plants showed a reduction of the total SOD activity in all the plants tested (Fig. 4 B). Some symptoms, such as reduction of photosynthetic pigments, protein content, plant height (data not shown), leaf area (Fig. 2) and especially of the total SOD, induced by oxidative stress (Fig. 4 B) are most frequently associated with accelerated senescence.

A comparison of the isozyme pattern of SOD in the gel revealed four isoforms generated by *S. commersonnii*. All frost-resistant hybrids and *S. tuberosum* 'Matilda', and 'Venta' generated nine isoforms, but, according to Martinez et al. [1], *S. tuberosum* generated six SOD isoforms. Our results demonstrated that UV-B did not induce new isoforms in the hybrids, but changed the activity of separate SOD isozymes; some isoforms were stimulated, while others were suppressed. Under the influence of UV-B upon *S. commersonii*, reduction of the activity of one SOD isoform was very evident (Fig. 5, lane 2). However, the total SOD activity after UV-B irradiation remained higher than in the control plant (Fig. 4 A). Probably the oxidative stress had induced different expression of SOD genes. In potato leaves, the same UV-B dose stimulated the synthesis *de novo* of SOD isozyme [13]. In *Arabidopsis thaliana* [10], under UV-B induction the

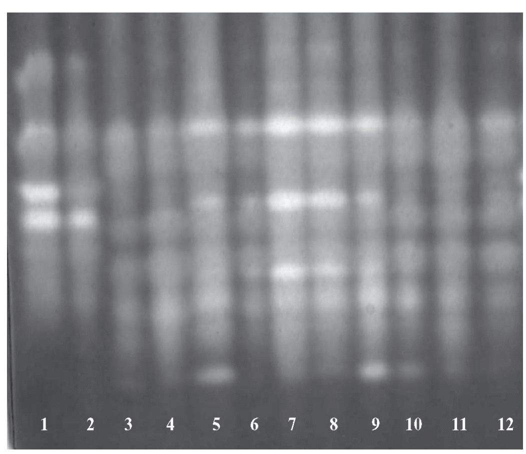

Fig. 5. Effect of UV-B irradiation on SOD isozymes of frost-resistant somatic of *S. commersonii* and *S. tuberosum* hybrids 2 weeks during 8 days of irradiation with UV-B 8 kJ m^{-2} d^{-1}. Lanes: 1, 2 – *S. commersoni*, 3, 4 – *S. tuberosum*; hybrids: 5, 6 – H 188; 7, 8 – H 269; 9, 10 – H 323; 11, 12 – H 515; lanes 2, 4, 6, 8, 10, 12 – control plants; lanes: 1, 3, 5, 7, 9, 11 – UV-B irradiated plants

total SOD activity increased also due to the preferential expression of Cu / Zn-SOD isozymes; their synthesis increased in both cytosol and plastids. There is some evidence that in transgenic potatoes permanently expressing Cu / Zn-SOD, an increased resistance to oxidative stress manifests by paraquat [14]. Further research will help to elucidate the exact SOD isoforms predetermining the total SOD activity in frost-resistant hybrids.

CONCLUSIONS

The total activity of SOD after UV-B impact increased in all the potato hybrids tested. However, SOD activity was higher in the frost-resistant hybrids than in the frost-sensitive *S. tuberosum* 'Matilda', 'Venta'. The SOD activity of the hybrids even exceeded the SOD level of the wild species *S. commersonii* both in the control plants and in plants exposed to UV-B. Therefore, the higher SOD activity under the impact of UV-B shows that UV-B irradiation induces an oxidative stress. SOD participates in detoxication of active oxygen forms because SOD isozymes take part in the oxidation protection mechanisms evoked by abiotic stress. The frost-resistant potato hybrids demonstrated an even higher tolerance level to UV-B irradiation and could better resist oxidative stress and recover at a faster rate than frost-sensitive *S. tuberosum.*

Received 27 November 2006
Accepted 21 February 2007

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UV-B POVEIKIS SOMATINIŲ BULVIŲ HIBRIDŲ ANTIOKSIDACINĖS SISTEMOS FERMENTUI – SUPEROKSIDO DISMUTAZEI (SOD)

Santrauka

Tiriant augalų atsaką į UV-B stresą ir įvertinant antioksidacinio fermento SOD vaidmenį adaptacijos mechanizmuose buvo ištirti *S. tuberosum* ir *S. commersonii* atsparūs šalnoms somatiniai bulvių hibridai ir jų tėvinės formos. Tyrimai rodo, kad dėl UV-B poveikio sumažėjo lapų plotas, sausa jų biomasė, chlorofilo a ir b koncentracija, tačiau antioksidacinis fermento SOD aktyvumas padidėjo visuose tirtuose augaluose. Atspariuose šalnoms bulvių hibriduose SOD aktyvumas buvo didesnis negu jautriuose šalnoms *S. tuberosum* 'Matilda' augaluose. Atsparūs šalnoms bulvių hibridai labiau toleravo kitą abiotinį veiksnį – UV-B spinduliuotę. Padidėjęs SOD aktyvumas po UV-B spinduliuotės vertintinas kaip augalo adaptacinis atsakas į UV-B sukeltą oksidacinį stresą.