ANTIOXIDATIVE ENZYME ACTIVITY IN FIELD-GROWN RED RASPBERRY AND BLACKBERRY PLANTS DURING EXPOSURE TO WATER DEFICIT AND DIFFERENT LIGHT INTENSITIES

ACTIVITATEA ENZIMELOR ANTIOXIDATIVE ÎN PLANTE DE ZMEUR ŞI MUR CRESCUTE ÎN CÂMP ÎN TIMPUL EXPUNERII LA DEFICIT HIDRIC ŞI DIFERITE INTENSITĂȚI DE LUMINĂ

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Abstract. *To control the level of reactive oxygen species and to protect the cells, plants possess a number of low molecular mass antioxidants and enzymes scavenging ROS (reactive oxygen species). Under normal conditions, the production and scavenging of ROS are well regulated. However, under stressful conditions such as drought ROS formation might be in excess of antioxidant scavenging capacity, thus creating oxidative stress. This work investigated the effects of water deficit on the activity of foliar antioxidative enzymes—superoxide dismutase (SOD), catalase (CAT), guaiacol (POD) and ascorbate (APX) peroxidases in three raspberry (Cayuga, Opal and Ruvi) and two blackberry (Thornfree and Lochness) cultivars grown under full sunlight (100% solar light, HL) and low light (25% solar light, LL) conditions in the experimental field. Each category was divided into 2 groups. One group was used as control and maintained in optimal water conditions by supplementary irrigations; the second group was not irrigated and potentially exposed to water stress. Under HL and water deficit conditions CAT and POD activities increased in both berry species irrespective of the cultivar. In contrast, the APX activity was up-regulated in raspberry and down-regulated in blackberry while SOD response to water deficit was dependent on cultivar. Under LL and water deficit conditions the POD activity increased in all raspberry cultivars while it declined in blackberry. In general, the activities of antioxidative enzymes were higher in HL-grown plants than in LL-grown plants during exposure to water deficit. The cultivars Cayuga (raspberry) and Thornfree (blackberry) showed more efficient antioxidative characteristics which could provide better protection against oxidative stress in leaves under water limited conditions.*

Key words: *raspberry, blackberry, water deficit, antioxidative enzymes, light intensity.*

Rezumat. *Pentru a controla nivelul de specii reactive de oxigen (ROS) și pentru a proteja celulele, plantele posedă antioxidanti și enzime antioxidative. În condiții normale, producția și inactivarea speciilor active de oxigen sunt bine reglementate. Cu toate acestea, în condiții de stres, cum ar fi seceta, formarea de ROS ar putea fi în exces față de capacitate antioxidantă, creând astfel stresul oxidativ. Aceasta lucrare a investigat efectele deficitului de apă asupra*

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activității enzimelor antioxidative foliare-superoxid dismutaza (SOD), catalaza (CAT), guaiacol peroxidaza (POD) și ascorbat peroxidaza (APX), în trei soiuri de zmeur (Cayuga, Opal si Ruvi) și două soiuri de mur (Thornfree si Lochness) cultivate în condiții de radiație solară de 100% (HL) și 25% (LL), în câmpul experimental. Fiecare categorie a fost împărțită în 2 grupe. Un grup a fost folosit ca şi control fiind mentinut în condiții optime de apă prin irigare suplimentară, al doilea grup nu a fost irigat și potențial expus la stresul hidric. In conditii de HL si deficit de apă activitățile CAT și POD au crescut în ambele specii, indiferent de soi. În schimb, activitatea APX a fost stimulată în zmeur şi inhibată în mur, iar răspunsul enzimei SOD la deficitul de apă a fost dependent de genotip. În condiții de LL si deficit de apă activitatea POD a crescut în toate soiurile de zmeur, în timp ce a scăzut în cele de mur. În general, activitatea enzimelor antioxidante a fost mai mare în plantele cultivate la HL decât în cele crescute la LL în timpul expunerii la deficitul de apă. Soiurile Cayuga (zmeur) și Thornfree (mur) au avut caracteristici antioxidante mai eficiente, fapt care ar putea oferi o mai bună protecție împotriva stresului oxidativ instalat în frunze ca urmare a deficitului de apă din sol.
Cuvinte cheie: *zmeur, mur, deficit hidric, enzime antioxidative, intensitatea luminii*

INTRODUCTION

Drought can cause oxidative stress and lead to the formation of reactive oxygen species (ROS). To control the level of reactive oxygen species and to protect the cells, plants possess a number of low molecular mass antioxidants and antioxidative enzymes. The ability for rapid upregulation of these enzymes was closely correlated with drought tolerance in different species (Wang et al., 2009).

The areas in Northeastern Romania, where raspberries and blackberries can be extensively grown are falling under water stress condition. Thus, identification of highly productive cultivars with better tolerance to water limited regimes is extremely important. This work investigated the effects of water deficit on the activity of foliar antioxidative enzymes: superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD) and ascorbate peroxidase (APX) in several raspberry and blackberry cultivars grown under two different light intensities in the experimental field in order to evaluate their tolerance to water deficit for future breeding programs.

MATERIAL AND METHOD

Three cultivars of raspberry (*Rubus idaeus* L.) Ruvi, Opal and Cayuga and two cultivars of blackberry (*Rubus fruticosus* L.) Thornfree and Lochness were cultured in June 2011 at the experimental station "V. Adamachi" from Iasi, Romania. The orchard was divided in two categories: HL, plants grown in full sunlight and LL, plants grown in shade conditions by covering them with a net which retained 75% sunlight (LL). Both HL and LL plants were grown under natural conditions of precipitations but each category was divided in two lots: one that was maintained in well watered conditions (85% FC) by supplementary irrigation (control, C) and the other one that was non-irrigated and thus exposed to water deficit. Samples (leaf tissues approximately from 5 plants/experimental variant) were taken after two months of plant growth under the above conditions.

Enzyme extracts were prepared using the method described by Vitória et al. (2001) with some modifications. The quantification of total soluble proteins was performed using the method of Bradford (1976). Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Rao and Sresty, 2000). Catalase (CAT) activity was determined using the method described by Aebi (1983). POD activity was estimated according to Putter (1983). Ascorbate peroxidase (APX) activity was determined using the method described by Nakano and Asada (1981). All spectrophotometric measurements were made using a T70 UV/VIS spectrophotometer (PG Instruments Ltd., London, UK).

Each analysis consisted of triplicate measurements of each sample and data were averaged over the three measurements. All data were subjected to t-test analysis at the $P<0.05$ probability level using SPSS 20.0 software package. The results are given as percentage of the corresponding well-watered controls.

RESULTS AND DISCUSSIONS

Changes in the activities of antioxidative enzymes in raspberry. Superoxide dismutases (SOD, EC 1.15.1.1) are enzymes that catalyze the dismutation of superoxide (O^{2-}) into oxygen and hydrogen peroxide. In this study we determined only the total SOD activity in leaf samples. The activity of SOD in non-irrigated raspberry plants declined in Opal and Ruvi. In Opal the light intensity did not influence this response. Here, SOD activity recorded values that were 50% lower than the corresponding control values, irrespective of the light treatment. However in Ruvi, the activity of SOD showed a larger decrease in HL (70%) than in LL conditions (50%). In contrast, SOD activity increased in Cayuga when plants were grown under HL conditions and did not change under LL conditions (Fig. 1).

Catalase (CAT, EC 1.11.1.6) is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. CAT activity increased in plants grown under HL conditions with Ruvi and Opal showing the largest increases. Under LL conditions it decreased slightly in Ruvi and Cayuga and approximately 50% in Opal (Fig. 1).

Ascorbate peroxidases (APX, EC 1.11.1.11) are enzymes that detoxify peroxides such as hydrogen peroxide using ascorbate as a substrate. Under HL conditions, APX increased by 50% in Opal and Cayuga and only 20% in Ruvi. In plants grown under LL conditions, APX activity in Ruvi and Cayuga showed values that were higher by 150% and 50%, respectively than those of well-watered controls. The values obtained for Opal were slightly lower than control (Fig. 1).

Guaiacol peroxidase (POD, EC 1.11.1.7) reduces H2O2 to water using various substrates as electron donors. It increased largely in all cultivars irrespective of the light treatment. The highest values were obtained in Opal+LL followed by Ruvi+HL.

*Changes in the activities of antioxidative enzymes in blackberry***.** The activity of SOD decreased in Lochness plants grown under HL conditions by 40%

while it did not change in plants grown under LL. In Thornfree, SOD activity increased under both light conditions mostly at HL (Fig. 2).

The alterations of CAT activity were influenced by light intensity rather than by genotype. It increased by 70% in plants grown under HL conditions and did not change in plants grown under LL. APX activity increased highly in Lochness+LL while it decreased in Thornfree and Lochness+HL. POD increased in both cultivars exposed to HL, mainly in Lochness while it decreased in Thornfree+LL or did not change in Lochness+LL (Fig. 2).

Similarities and differences in the activities of antioxidative enzymes. Similarities and differences in the activities of the oxygen-scavenging enzymes, SOD, POD, APX, and CAT were detected among raspberry and blackberry cultivars exposed to soil water deficit. The differences were imposed by light conditions and genotype. In general, the activities of antioxidative enzymes were higher in HL-grown plants than in LL-grown plants suggesting that high light combined with water deficit resulted in enhanced production of ROS by the photosynthetic apparatus because these conditions limit the availability of CO2 for the dark reaction, leaving oxygen as one of the main reductive products of photosynthesis.

Under HL+water deficit conditions CAT and POD activities increased in both berry species irrespective of the cultivar. In contrast, the APX activity was up-regulated in raspberry and down-regulated in blackberry suggesting the presence of species-dependent regulatory mechanisms of APX. SOD response to water deficit was dependent on genotype.

Among berry cultivars, Cayuga and Thornfree showed increased activities of all categories of enzymes (SOD, CAT and POD) which may contribute to a higher degree of tolerance of these cultivars to the growth conditions. In Opal, Ruvi and Lochness SOD down-regulation could result in the overproduction of the H2O2 and O2.- whereas increased activities of catalase and peroxidases suggests high demands of H2O2 quenching.

Under LL+water deficit conditions the POD activity increased in all raspberry cultivars while it declined in blackberry. The fact that POD is up-regulated in raspberry irrespective of the light treatment and genotype suggests a response exclusively induced by water deficit. In contrast, the activity of POD in blackberry seems to be depended on light intensity, this enzyme being up-regulated at high light.

In conclusion, the above mentioned enzymes act as a system. Some of them seem to be under different regulatory mechanisms and/or they are compartmentalized in different organelles. To further elucidate the role of these enzymes in tolerance of raspberry and blackberry plants to water deficit, their cell localization needs to be determined.

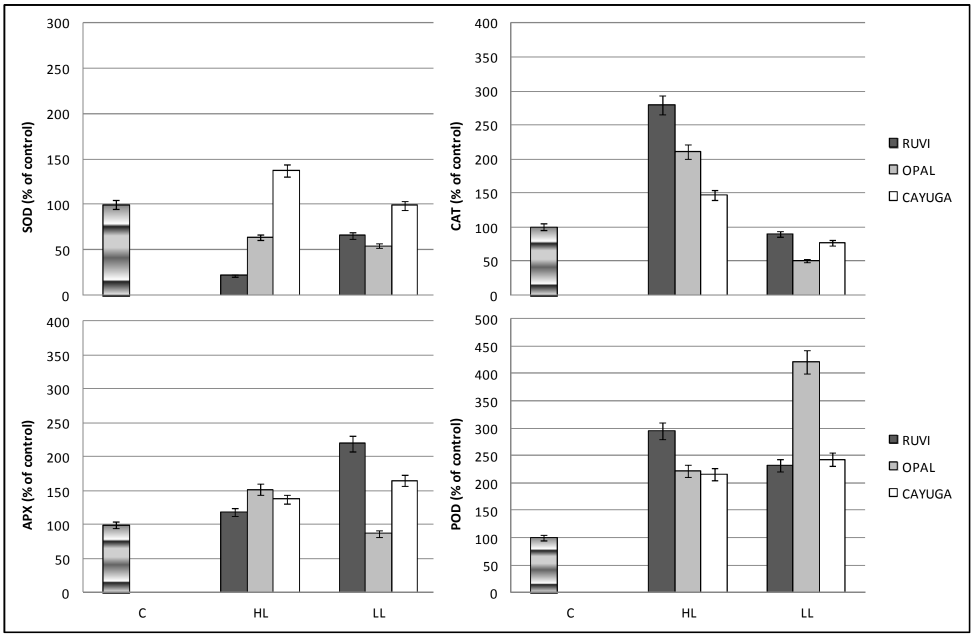

Fig. 1 - The activities of key antioxidative enzymes (SOD, CAT, APX and POD) in three red raspberry cultivars grown without irrigation under different light intensities. The values are expressed as percentage of irrigated control plants (C)

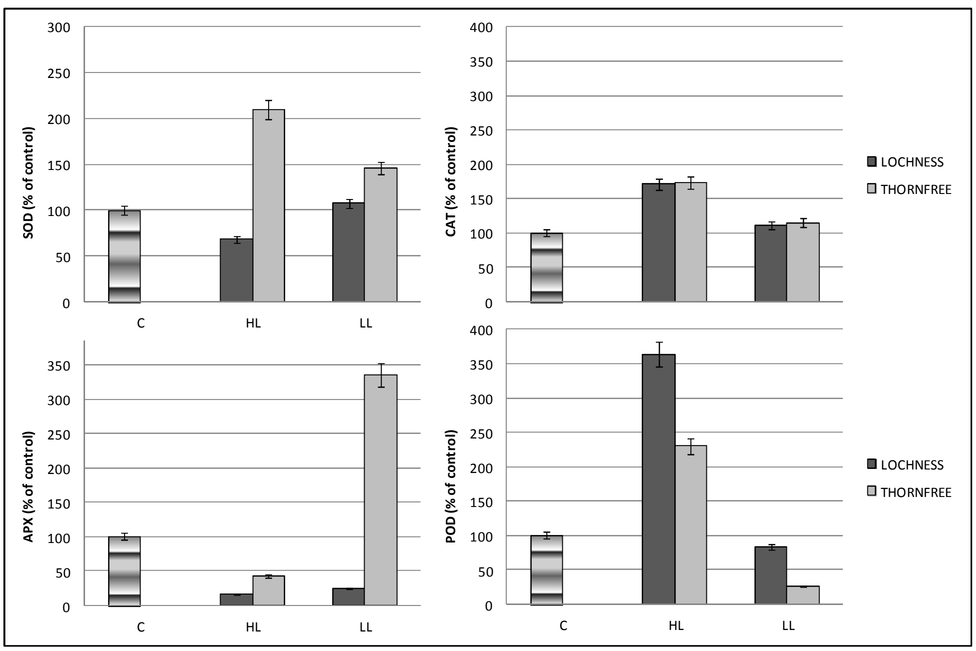

Fig. 2 - The activities of key antioxidative enzymes (SOD, CAT, APX and POD) in two blackberry cultivars grown without irrigation under different light intensities. The values are expressed as percentage of irrigated control plants (C)
(for details, see Materials and Methods)

CONCLUSIONS

1. The activities of antioxidative enzymes were higher in HL-grown plants than in LL-grown plants during exposure to water limited conditions.

2. Under water deficit conditions, HL increased CAT and POD activities in both berry species irrespective of the cultivar.

3. APX and SOD response to a combination of high light and water deficit is influenced by species and cultivar, respectively.

4. During water deficit POD is up-regulated in raspberry plants irrespective of the light treatment and genotype. In blackberry its activity is dependent on light intensity.

5. The cultivars Cayuga (raspberry) and Thornfree (blackberry) exhibited efficient antioxidative characteristics which could provide better protection against oxidative stress in leaves under water limited conditions.

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