BIOCHEMICAL AND FUNCTIONAL ALTERATIONS IN THE PHOTOSYNTHETIC APPARATUS OF *RUBUS IDEAUS* L. IN RESPONSE TO WATER AND LIGHT ENVIRONMENT

ALTERĂRI BIOCHIMICE ŞI FUNCŢIONALE ÎN APARATUL FOTOSINTETIC DE *RUBUS IDEAUS* L. CA RĂSPUNS LA LUMINĂ ŞI CANTITATEA DE APĂ DIN SOL

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Abstract. *In higher plants, the CO_2 availability and light intensity are liable to be limiting under conditions of water stress, due to the closure of stomata and photoinhibition. Under such conditions, cells are likely to experience oxidative stress, due to the formation of reactive oxygen species associated with the absorption of light by chlorophylls. The aim of this work was to investigate the effects of water and light on the functional and biochemical parameters of photosynthetic apparatus in two red raspberry cultivars (Opal and Ruvi) cultured in field conditions. Plants have been divided into two categories; one category was used as control and maintained in optimal water conditions by irrigation; a second category was not irrigated, thus exposed to water deficit. In each category, 50% plants were exposed to 100% sunlit (HL) and 50% plants were shaded and exposed to 25% sunlit (LL). The functionality of photosynthetic apparatus after dark and light adaptation was assessed through chlorophyll fluorescence measurements using Handy Pea. Chlorophylls and carotenoids were also quantified. The results showed that some parameters had a higher degree of sensitivity to the experimental conditions than others. From all photosynthetic parameters, the performance index (Pi) showed the largest variations among plants from different experimental categories.*
Key words: *raspberry, water deficit, JIP test, chlorophylls*

Rezumat. *În plantele superioare, disponibilitatea CO_2 și intensitatea luminii pot deveni factori limitativi ai fotosintezei în condiții de stres hidric, ca urmare a închiderii stomatelor și fotoinhibitiei. În aceste condiții, celulele sunt susceptibile de a experimenta stresul oxidativ, datorită formării speciilor reactive de oxigen asociate cu absorbția luminii de către clorofile. Scopul acestei lucrări a fost acela de a investiga efectele apei și intensitatii luminii asupra parametrilor funcționali și biochimici ai aparatului fotosintetic în doua cultivare de zmeur (Opal si Ruvi) cultivate în condiții de camp. Plantele au fost împărțite în două categorii: o categorie a fost folosita ca si control fiind mentinuta în condiții optime de apă prin irigare; a doua categorie a fost neirigata si deci expusa stresului hidric. În fiecare categorie, 50% plante au fost expuse la 100% radiatie solara (HL) și 50% plante au fost umbrite și*

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expuse la 25% radiatie solara (LL). Funcționalitatea aparatului fotosintetic, după adaptarea la intuneric si lumina a fost evaluată prin măsurarea fluorescenței clorofilei folosind HandyPea. Clorofilele si carotenoizii au fost, de asemenea, cuantificate. Rezultatele au aratat ca unii parametri au avut un grad mai mare de sensibilitate la condițiile experimentale decât altii. Dintre toți parametrii fotosintetci investigati, indicele de performanta (Pi) a aratat cele mai mari variații între plantele din diferite categorii experimentale.
Cuvinte cheie: *zmeur, deficit hidric, JIP test, clorofile*

INTRODUCTION

Raspberries (*Rubus ideaus* L.) need an abundant supply of water at all times and, therefore, water deficit may drastically affect plant growth and development. One of the first physiological responses to water limitation in plants is stomatal closure which results in the decrease in photosynthetic carbon assimilation and may imbalance the photosynthetic electron transfer. If photosynthesis is inefficient, excess light energy must be dissipated to avoid damaging the photosynthetic apparatus. Energy can be dissipated as heat (non-photochemical quenching) or emitted as chlorophyll fluorescence.

A number of studies showed that chlorophyll fluorescence measurements could be used to estimate, rapidly and non-invasively, the responses of plants to abiotic stress including drought. Moreover, using this method, the effect of stress in plants can be detected before any symptoms can be observed visually (Živčák et al., 2008). Recently, chlorophyll fluorescence was used to measure heat tolerance for a segregating population of field-grown raspberries (Molina-Bravo et al., 2011) or for evaluation of drought stress in strawberry (Razavi et al., 2008). The aim of this work was to investigate the effects of water and light intensity on the functional and biochemical parameters of photosynthetic apparatus in two red raspberry cultivars (Opal and Ruvi) cultured in field conditions.

MATERIAL AND METHOD

Two raspberry (*Rubus idaeus* L.) cultivars, Ruvi and Opal were cultured in June 2011 at the experimental station “V. Adamachi” from Iasi, Romania. After a month of acclimation to field conditions 50% of plants were covered with a net retaining 75% of the solar radiation (LL). The other half of plants were not covered, thus exposed to full sunlight (HL). All plants were irrigated until the field capacity reached 90%. At this time (T0) we measured photosynthesis and collected leaf samples for pigment analysis. After that time half of plants of each light treatment were maintained well watered by irrigation (90% FC) and used as controls, and the other half plants were subjected to water deficit treatment by withholding irrigation until the end of experiments. Leaf samples and photosynthesis measurements were taken after 2 (T1), 5 (T2), and 10 (T3) weeks from T0.

To measure the photosynthetic pigments, the leaves were thoroughly homogenized in chilled 80% acetone in the dark at 4^0C. The homogenate was centrifuged at 4500 rpm for 10 min. The supernatants were collected and the absorbances were read at 663, 646 and 470 nm using a T70 UV/VIS spectrophotometer (PG Instruments Ltd., London, UK). The photosynthetic pigments were calculated following the equations of Lichtenthaler (1987).

Chlorophyll a fluorescence was measured using HandyPea (Hansatech Ltd., Norfolk, UK). The transients were induced by a red light (peak at 650 nm) of 3000 μmols $m^{-2}s^{-1}$ provided by an array of six light-emitting diodes (for details, see Strasser et al., 1995). The leaves were dark-adapted for 30 min before the fluorescence measurements. Excitation of leaves with red actinic light resulted in the rise of Chl fluorescence from the initial O-level (F0) to the maximum P-level (Fm) with two intermediate steps J and I. The fluorescence signal at 50 μsec after the onset of illumination was considered as F0 (Strasser and Strasser, 1995). The J step was the fluorescence measured at 2 ms while the I step was the fluorescence value recorded at 30 ms. The variable fluorescence, Fv (the difference between the initial fluorescence, F0, and the maximal fluorescence, Fm in dark adapted leaves) was used to calculate the Fv/Fm ratio. Data from fluorescence measurements were analyzed using the JIP-test. Several parameters such as absorbance per reaction centers (ABS/RC), energy dissipation per reaction center (DIo/RC) and the performance index (Pi) were calculated according to Strasser and Strasser (1995). 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 the difference (Δ) between the values obtained for irrigated and non-irrigated plants.

RESULTS AND DISCUSSIONS

Alterations induced by water deficit in the fluorescence parameters. The Fv/Fm ratio is a measure of the photosynthetic efficiency of a dark-adapted sample. An Fv/Fm value in the range of 0.79 to 0.84 is the approximate optimal value for many plant species, with lower values indicating plant stress (Maxwell and Johnson, 2000). During experiments, the Fv/Fm values maintained at 0.78±0.05 irrespective of measurement time, treatment or cultivar which means that PSII functionality was not affected (Fig. 1).

The ABS/RC describes the absorption flux per RC and gives information on antenna size. During T0-T2 there were no differences in the ABS/RC between control and non-irrigated plants in Ruvi+HL. In contrast the ΔABS/RC had negative values in T3 fact that suggests an increase in antenna size in non-irrigated plants probably due to the cumulative effects of water deficit and a reduction in light intensity. A similar situation was found in Opal+HL. An enlargement in antenna size was also found in non-irrigated plants grown under LL conditions but at different times (T1 in Opal and T2 in Ruvi) (Fig. 1).

The heat dissipation capacity (DI0/RC) indicates the rate of the total dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs. Dissipation in this context refers to the loss of absorbed energy through heat, fluorescence and energy transfer to other systems (Strasser et al., 2004). In our study DI0/RC was higher in non-irrigated plants, mainly at T2 with Ruvi showing the lowest ΔDI0/RC values. The loss of fluorescence at T2 might be due to the cumulative effects of water and temperature. However, it was not accompanied by changes in the Fv/Fm ratio and thus it can be seen as a protective mechanism of PSII functionality (Fig. 1).

The performance index Pi may be used to estimate the degree of plant vitality because it includes three independent parameters: the density of fully active reaction centers (RCs); the efficiency of electron movement by trapped exciton into the electron transport chain beyond the QA; and the probability that an absorbed photon will be trapped by RCs (Strasser et al. 2004). Figure 1 depicts that soil water and light conditions highly influenced the performance index. The ΔPi had positive values that continusly increased from T0 to T3 in both cultivars maintained under HL conditions (Fig. 1).

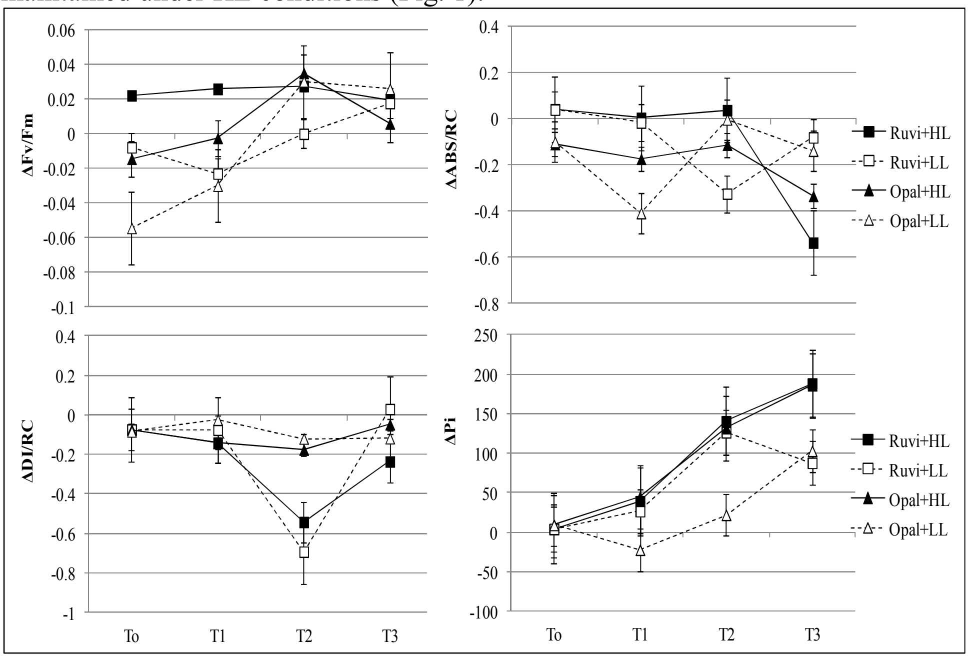

Fig. 1 - Differences (Δ) in the maximum quantum yield of PSII photochemistry (Fv/Fm), absorbance per reaction centers (ABS/RC), dissipation of excess energy (DI/RC) and performance index (Pi) between irrigated and non-irrigated plants during exposure of two raspberry cultivars to water deficit under full sunlight (HL) and reduced sunlight (LL) conditions

However, in Opal control plants grown under LL had lower Pi values than those grown under HL. Moreover, water stressed plants grown had the same photosynthetic performance with control plants. These results suggest that Opal is more sensitive to LL than HL but in conditions of water deficit it also became sensitive to HL (Fig. 2), due to a reduction in the density of active reaction centers and electron transport rate (data not shown). A different situation was found in Ruvi where from T0 to T2 control plants had the same Pi irrespective of the light treatment. These data suggest that the Pi response to water deficit in Ruvi was not influenced by light intensity. However in T3 the photosynthetic performance of LL grown plants was higher than in HL-grown plants due to changes in the proportion of active reaction centers (data not shown) (Fig. 2).

It is well known that chlorophyll content is one of the major factors affecting photosynthetic capacity. Alterations in chlorophyll content of plant under drought stress have been observed in different plant species and they depended on stress intensity and duration (Kyparissis et al., 1995). In our study we found that the amount of total chlorophylls was higher in non-irrigated plants because ΔChls had negative values among treatments. Prolonged water deficit increased even more the amount of total chlorophylls in plants maintained under HL conditions and therefore the corresponding ΔChls had lower values in T2 than in T1 (Fig. 3). Increases in chlorophyll content under drought stress were also reported by Mensah et al. (2006) for sesame or Beeflink et al. (1985) for onion. A similar pattern was found for carotenoids which increased in plants exposed to water deficit (data not shown).

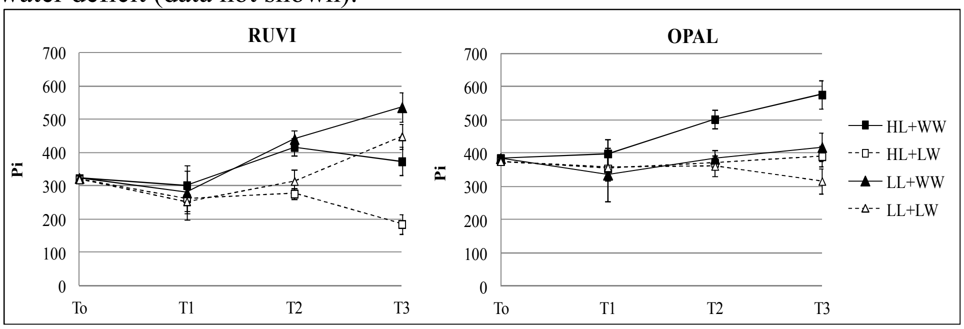

Fig. 2 - Alterations in the performance index (Pi) during exposure of two raspberry cultivars to well-watered (WW) and water deficit (LW) conditions in full (HL) and reduced (LL) sunlight

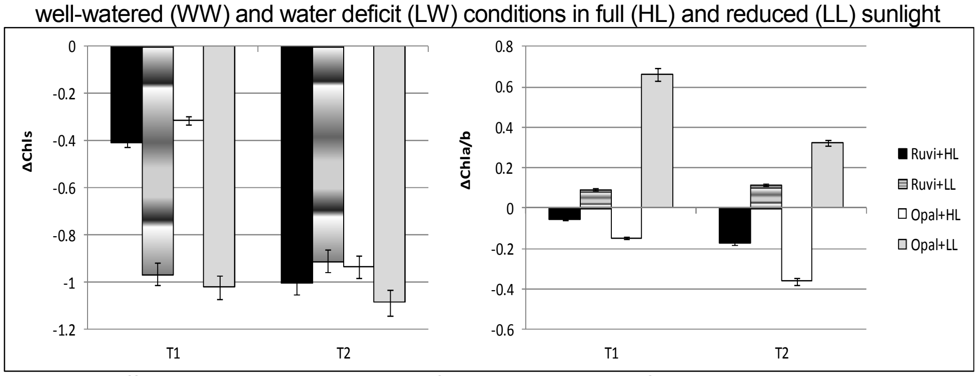

Fig. 3 - Differences (Δ) in the amount of total chlorophylls (Chls) and chlorophyll a/b ratio (Chl a/b) between irrigated and non-irrigated plants during exposure of two raspberry cultivars to water deficit under full sunlight (HL) and reduced sunlight (LL) conditions

The Chl a/b ratio showed large variations among treatments. In T1 as well as T2 the ΔChl a/b had negative values in plants grown under HL conditions and positive in those grown under LL which means that water deficit decreased the Chl a/b ratio in LL plants but increased it in HL plants (Fig. 3). Since Chl a/b ratio is an indicator of light harvesting size (Givnish, 1988) one may assume that antenna increased under a combination of LL and water deficit. Further investigations are needed to understand the role of light during acclimation of raspberry plants to water deficit.

CONCLUSIONS

1. Soil water deficit induced changes in the functionality of photosynthetic apparatus that culminated with the decrease of photosynthetic performance.

2. The response of cultivars to water deficit was modulated by light intensity. In general, Opal was more sensitive to shade conditions than Ruvi.

3. Water deficit did not alter the chlorophyll content but it induced changes in the chlorophyll pattern.

4. Pi can be used as screening tool for drought tolerance in raspberry.

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