WATER DEFICIT AND LIGHT INTENSITY EFFECTS ON THE ACCUMULATION OF TOTAL PHENOLICS AND ANTHOCYANINS IN SEVERAL RASPBERRY AND BLACKBERRY CULTIVARS

INFLUENTA DEFICITULUI HIDRIC SI A INTENSITATII RADIATIEI SOLARE ASUPRA ACUMULARII COMPUSILOR FENOLICI TOTALI SI A ANTOCIANILOR LA UNELE SOIURI DE ZMEUR SI MUR

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Abstract. *In field conditions, high light intensities often accompanied drought, which may significantly affect plant growth and development. Both water and light may decrease the capacity of plants for carbon assimilation by stomatal closure and photoinhibition. Angiosperms can prevent excessive light absorption by spatial leaves repositioning or photoprotective pigments synthesis such as anthocyanins. The aim of this work was to investigate the effects of water stress and light intensity on the accumulation of total phenolics and anthocyanins in several raspberries (Opal, Cayuga si Ruvi) and blackberry (Thornfree si Lochness) cultivars. The experiments were perfomed from June to October 2011. Plants have been subjected to two water conditions, irrigated and non-irrigated, and two light intensities 100% sunlit and 25% sunlit. In all cultivars, total phenolics content was sensitive to light conditions and tolerant to poor water conditions. A combination of water stress and low light intensities decreased the anthocyanin content in raspberry cultivars. Contrary, the accumulation of anthocyanins increased in all blackberry cultivars when exposed to water stress, irrespective of light conditions. The relative importance of these phenolic compounds to the protection of raspberry and blackberry plants against abiotic stress is discussed.*
Key words*:* *Rubus* sp., phenolics ,anthocyanins

Rezumat. *Adeseori, in cazul cultivarii plantelor horticole in camp, deficitul hidric este însotit de intensitāti luminoase mari iar această combinatie de factori abiotici poate conduce la scaderea capacitātii fotosintetice datorită inchiderii stomatelor si fotoinhibitiei. Angiospermele pot preveni absorbția în exces a luminii de care frunze prin repoziționarea lor spațială sau prin sinteza unor pigmenți fotoprotectori de tipul carotenoizilor sau a antocianilor. În această lucrare ne-am propus determinarea conținutului în polifenoli totali şi antociani din frunze la unele soiuri de zmeur (Opal, Cayuga şi Ruvi) şi mur (Thornfree şi Lochness), cultivate în câmp în condiții de apă şi lumină diferite. Experimentul s-a desfăşurat în perioada iunie-octombrie 2011. Plantele au fost cultivate în regim irigat şi neirigat cu 100% expunere la lumină şi umbrite la 25% lumina. Conținutul de polifenoli a fost influențat în mod semnificativ de condițiile de lumină şi mai puțin de regimul hidric la toate soiurile luate în*

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studiu. În schimb, interacțiunea celor doi factori abiotici influențeaza diferit acumularea de antociani, astfel incat în condiții de stres hidric conținutul de antociani în frunze scade la zmeur doar la plantele menținute la umbră. În schimb, la mur există o acumulare mai mare a antocianilor la plantele neirigate indiferent de condițiile de lumină.
Cuvinte cheie: *Rubus* sp., polifenoli, antociani

INTRODUCTION

In field conditions, water stress is often accompanied by excessive light which can significantly alter plant growth and development. The light energy that exceeds the assimilation abilities of the leaves may be caused by the high incidence of the solar radiation, as well as by the decline in photosynthetic carbon assimilation, resulting from stomatal closure. This excess energy causes an increase in the production of active oxygen species (ROS) (Mittler, 2006) which may lead to the inhibition of photosynthesis and growth processes (Lawlor and Cornic, 2002; Chaves et al*.,* 2003).

In order to prevent damage, plants have developed different protection mechanisms of the photosynthetic apparatus against excess light associated with tissue dehydration (Tuba et al*.,* 1996). Angiosperms can prevent the excess absorption of light through spatial repositioning of leaves or synthesis of some photoprotective pigments such as carotenoids or anthocyanins (Sherwin and Farrant, 1998). On the other hand, the stimulation of the plant antioxidant capacity is a highly efficient protection mechanism against the harmful effects of oxygen radicals (Sherwin and Farrant, 1998). Phenols, mainly antocyanins are chemical compounds with high antioxidative effects that play an important role in the adaptation of plants to abiotic stress factors (Smirnoff and Cumbes, 1989; Apel and Hirt, 2004; Kruk et al*.,* 2005).

MATERIAL AND METHOD

The experiment has been carried out in the experimental field of SDCV Adamachi, in 2011. Plant material was represented by three varieties of raspberry (Opal, Cayuga and Ruvi) and two of blackberry (Thornfree and Lochness). Plants were cultivated continuously since June in two distinct sunlight conditions. One group was given full 100% sunlight and the second group had the sunlight reduced by 75%. The reduction was obtained by net covering. Both variants were maintained in well watered conditions by supplementary irrigation and natural water flow (non-irrigated). After two months, leaf samples were harvested from 3 plants / variant in 3 repetitions / plant. The leaves were ground in liquid nitrogen and kept at -80^{0}C.

Determination of total phenolics:

100 mg ground tissue was extracted with 1 ml 60% acetone for 60 min at 25 ^{0}C on shaker. Total phenolics were quantified in extracts using the Folin–Ciocalteu protocol, as modified by Singleton and Rossi (1965). Gallic acid was used as a standard, and results were calculated as gallic acid equivalent (GAE) (mg/100 g dry weight basis).

Determination of anthocyanin content:

Anthocyanins were extracted by incubating 100 mg of grounded tissue overnight in 150 µL of methanol acidified with 1% HCl (v/v). Total anthocyanins were determined by measuring the A530 and A657 of the aqueous phase using a spectrophotometer. By subtracting the A657 from the A530, the relative amount of anthocyanin was calculated as OD/g.fw. (Neff & Chory, 1998).

All spectrophotometric assays were carried out with a T70 UV /VIS spectrophotometer (PG Instruments LTD)

RESULTS AND DISCUSSIONS

Plants can accumulate phenolic compounds under various stress conditions such as light, low temperature, hydric deficit (Sakihama et al. 2002).

Our data show that total phenolics are relatively stable among the experimental categories. However, we noticed an increase in the total phenolics content under water deficit in Opal and Cayuga plants cultured in 100% sunlight conditions. The water deficit did not alter total phenolics in Opal and Cayuga cultured in shade and water deficit conditions but it induced their decrease in Ruvi (fig. 1).

Under water deficit conditions, the accumulation of total phenolics was slightly stimulated in Thornfree, mainly in plants cultured in full sunlight conditions. Contrary, total phenolics decreased in Lochness, mainly in shaded plants (fig. 2).

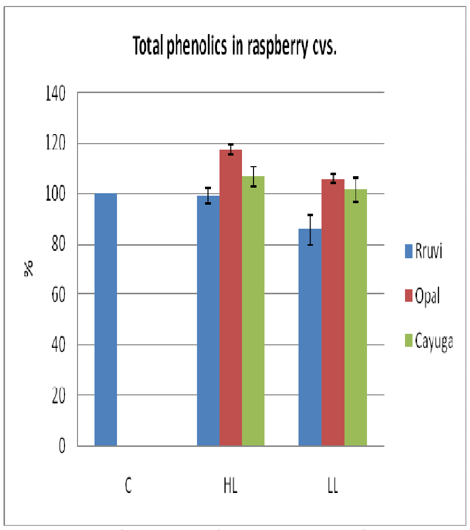

Fig. 1 – Influence of the water deficit on the total phenol content in 3 raspberry varieties cultivated under two light conditions

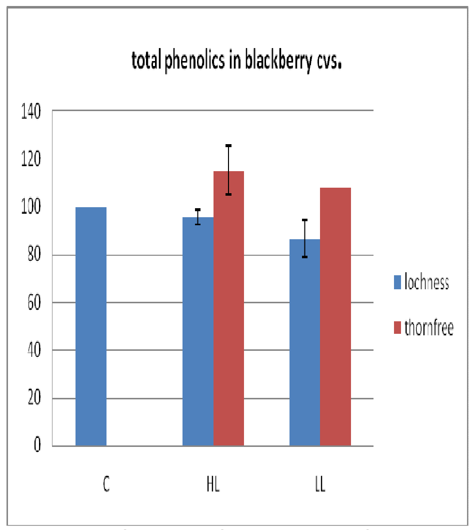

Fig. 2 – Influence of the water deficit on the total phenol content in 2 blackberry varieties cultivated under two light conditions

Anthocyanins are water-soluble pigments that belong to the family of flavonoids. They are also involved in defence against environmental stresses such as ultraviolet radiation, herbivores, drought and chilling (Chalker-Scott 1999, Hatier and Gould 2008). Close & Beadle (2003) have reviewed and discussed the ecophysiological roles of anthocyanins as antioxidants, compatible solutes in osmotic regulation, and photoprotectants against ultraviolet radiation and visible light.

Plant tissues containing anthocyanins are usually resistant to drought (Chalker-Scott 1999). For example, the drought tolerant resurrection plants highly accumulate anthocyanins during dehydration (Sherwin and Farrant 1998). A possible mechanism of anthocyanin-induced drought resistance is related to anthocyanins ability to stabilize the water potential (Choinski and Johnson 1993, Chalker-Scott 2002).

Under water deficit conditions, we found an increase in anthocyanins in Ruvi (18%) and Opal (10%) plants exposed to 100% sunlight, while it decreased in the plants maintained in shade conditions. Contrary, anthocyanins increased in Cayuga (18%) plants that received only 25% solar light. Our data suggest that light intensity is a factor that influences the sensitivity of these cultivars to water stress. Both Opal and Ruvi cultivars were less sensitive to water deficit when exposed to 100% sun light conditions, while Cayuga is more tolerant to water deficit when exposed to low light intensities (fig. 3).

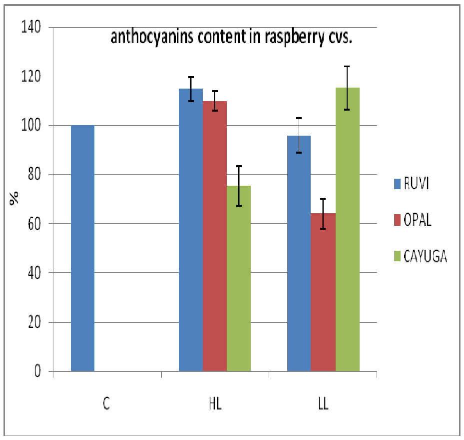

Fig. 3 - The influence of the water deficit on the anthocyanin content for some raspberry varieties cultivated under two light conditions

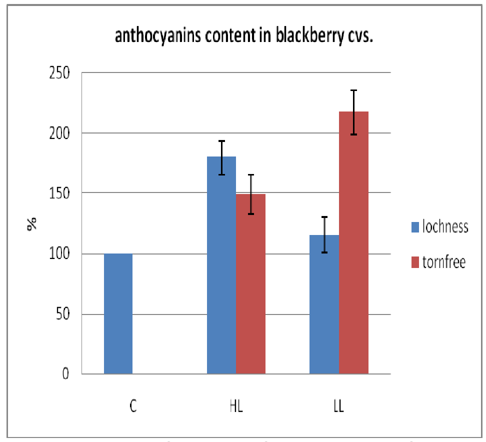

Fig. 4 - The influence of the water deficit on the anthocyanin content for some blackberry varieties cultivated under two light conditions

Konczak-Islam et al. (2003) reported at sweet potato that anthocyanin constituent was correlated with phenolic compounds content and their antioxidant activity. In our experiments case this challenge has been confirmed only for the blackberry species. For the raspberry the anthocyan content varies independently to that of the polyphenols.

In blackberry the accumulation of anthocyanins under water deficit conditions was influenced by light intensity and genotype. Under full sun light, both cultivars increased their anthocyanin content by 50-60%. Under low light conditions, the anthocyanin accumulation increased by 110% in in Thornfree and only 10% in Lochness (fig. 4).

The role of anthocyanins in vegetative tissues such as leaves is still under debate. The basic question is whether they are directly involved in stress responses or just assist in plant defense (Hatier and Gould 2008). For instance, anthocyanins are suggested to protect chloroplasts from excess irradiance due to their ability to absorb light between 400 and 600 nm, although carotenoids are more effective in this respect (Gould et al. 2000). Our data demonstrate that anthocyanins accumulation increased under water deficit conditions mainly in hight light conditions. This increase suggests that anthocyanins can be involved in photoprotection under direct drought stress, which is in accordance with previous investigations (Gould et al. 2000, Hoch et al. 2001, Close and Beadle 2003, Merzlyak et al. 2008).

CONCLUSIONS

1. The water deficit leads to the accumulation of total polyphenols in raspberry and blackberry cultivars under high light conditions.

2. Under water stress, there are genotypic variations in anthocyanin accumulation in response to light intensity.

3. The accumulation of anthocyanins under water deficit and high light condition suggests that these compounds may play a photoprotective role.

Acknowledgement. *The present contribution was supported by the UE-funding grant POSCCE-A2-O2.1.2-2009-2 ID.524, cod SMIS-CSNR 11986.*

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