DIFFERENTIAL EXPRESSION OF PHENYLPROPANOID BIOSYNTHETIC GENES IN RED RASPBERRY DURING WATER DEFICITS

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Introduction

Red raspberry fruits are an important dietary source of antioxidant compounds, in particular, polyphenols, which are famous for their health benefits. However, little is known about how water scarcity might affect phenylpropanoid gene expression in red raspberry.

In the present work, the effects of progressive water stress on three red raspberry (*Rubus ideaus* L.) cultivars (Opal, Cayuga and Ruvi) were investigated in a greenhouse environment. Transcripts of key phenylpropanoid genes (PAL, CHS, 4CL), the level of phenolic compounds, leaf relative water content and water use efficiency were evaluated in samples collected from plants exposed to different soil water levels such as full water supply (90% field capacity, FC), moderate water stress (50% FC), and severe water stress (35% FC).

Materials and Methods

Experimental conditions *Rubus* plants were divided in two lots and grown under greenhouse environment for 3 weeks. One lot (control) was maintained by irrigation at 90% FC. The other lot was exposed to water stress by withholding water until the field capacity (FC) decreased to 35%. The pots were kept at the above drought stress levels by weighting. Control plants were watered daily. Plant leaves were collected from three biological replicates ground to a fine powder in liquid nitrogen and stored at -80ºC to preserve full-length RNA.

RNA isolation and quantification. Total RNA extraction and purification was performed with Spectrum Plant Total RNA kit. RNA quality was verified by Agilent Bioanalyzer analysis using an RNA 6000 Nano Labchip kit.

cDNA synthesis and RT-PCR. RNA was reverse transcribed with SuperScript II Reverse Transcriptase kit (Invitrogen).The resulted first-strand cDNA was amplified using gene-specific primers designed from the transcribed region of each gene specific for *Rubus idaeus* spp., using Primer Express 1.5 software (Applied Biosystems, Darmstadt, DE). Primers were obtained for phenylalanine ammonia-lyase (pal1 and pal2), chalcone synthase (chs), 4-coumarate:coA ligase (cl1, cl2 and cl3), histone H3 and actin.

qRT-PCR analysis. Quantitative real-time PCR analysis was performed on the Rotor-Gene 6000 (Corbette) using MyTaqTMRedMix (Bioline).The temperature cycle used comprised 40 cycles at 95°C for 15 sec and 60°C for 1 min. To monitor PCR specificity a dissociation curve was performed. For the relative quantification of transcript levels, a modification of the comparative threshold cycle method was used. Relative transcript levels of the gene of interest (X) were calculated as a ratio to the histone H3 gene transcripts (U), as (1+E)–ΔCt, where ΔCt was calculated as (CtX–CtU). PCR efficiency (E) for each amplicon was calculated employing the linear regression method (Ramakers et al. 2003).

Relative water content (RWC). For the determination of RWC, the method of Barrs and Weatherley (1962) was used.

Water use efficiency (WUE). It was calculated as the ratio between photosynthesis and transpiration. Both parameters were measured with a portable IRGA (LiCl).

Chlorophyll fluorescence. Chl a fluorescence was measured using HandyPea (Hansatech Ltd., Norfolk, UK). The transients were induced by red light (650 nm) of 3000 µmols $m^{-2}s^{-1}$ provided by an array of six light-emitting diodes. The leaves were dark-adapted for 30 min before the measurements. Excitation of leaves with red actinic light resulted in the rise of Chl fluorescence from the initial O-level to the maximum P-level with two intermediate steps J and I.

Determination of total phenolics (TPH). The total phenolics were determined using the Folin-Ciocalteau reagent as reported by Javanmardi et al (2003).

Determination of total anthocyanins (ACY). Anthocyanin quantitation was performed in leaf samples by the pH differential method of Giusti and Wrolstad (2003). Values were expressed in terms of mg of anthocyanin/100 g FW.

Results

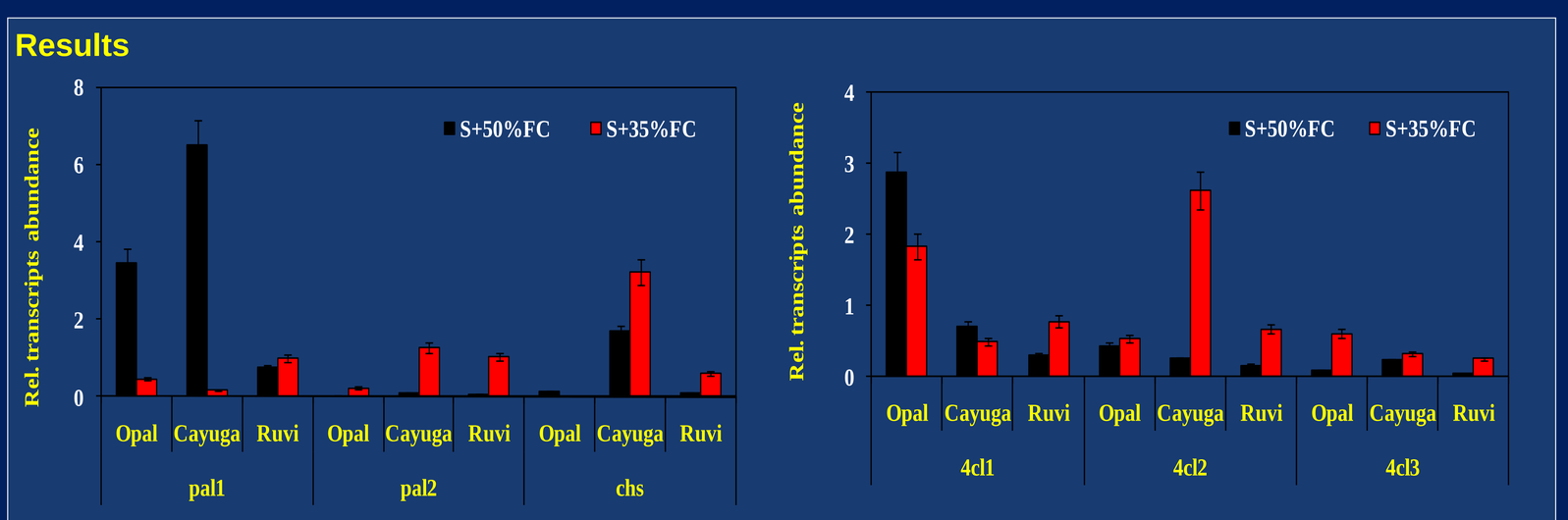

***Fig. 1**. Expression levels of phenylpropanoid genes in red raspberry cultivars during progressive drought. Relative transcript abundance of PAL, CHS and 4CL genes was determined using qRT-PCR assay. Values are given as the ratio between stress and control plants (n=4).*

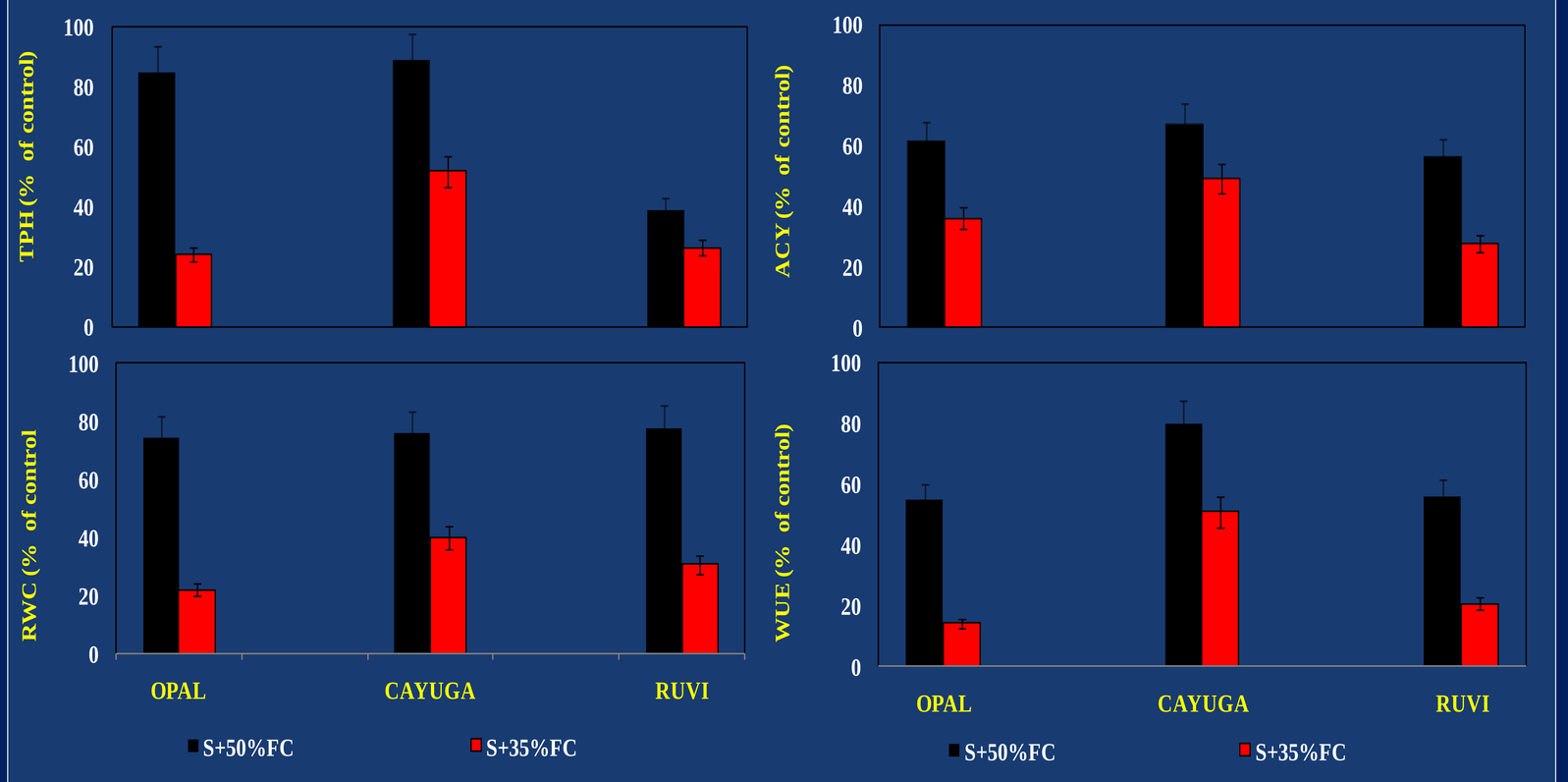

***Fig. 2**. Changes in total phenolics (TPH), anthocyanins (ACY), relative water content (RWC) and water use efficiency (WUE) in three red raspberry cultivars during progressive drought (n=5).*

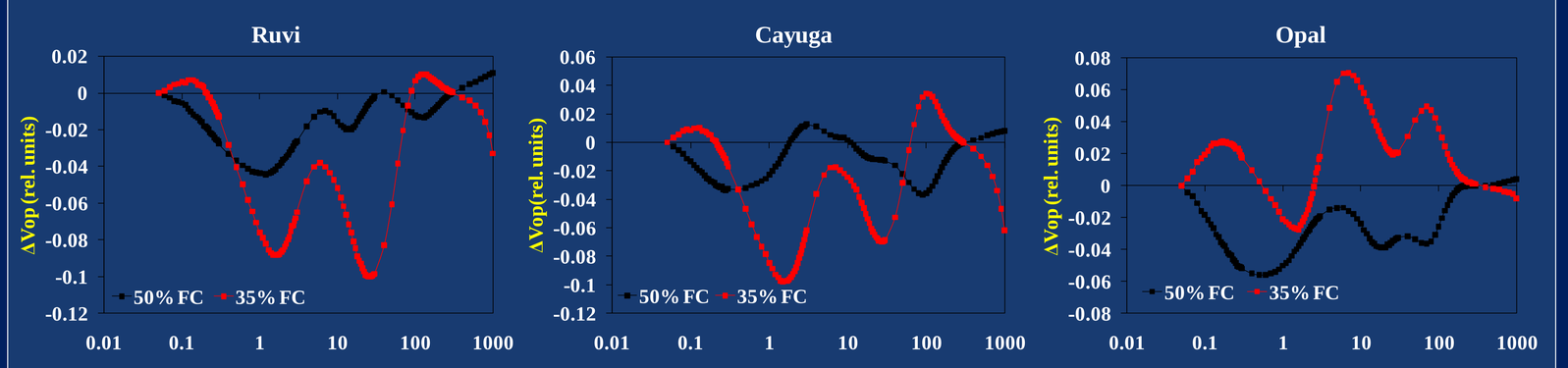

***Fig. 3.** Alterations in variable chlorophyll fluorescence under the influence of water stress in red raspberry cultivars. Values are given as the difference between variable fluorescence measured in control and stressed plants. Time (ms) is given on logarithmic scale on X-axis.*

Conclusions

1. The two PAL genes showed different patterns of expression during progressive drought. Moderate drought (50% FC) increased the transcription of PAL 1, whereas severe drought (35% FC) increased the transcription of PAL 2.

2. CHS and 4CL2 were up-regulated in Cayuga and down-regulated in Ruvi and Opal.

3. The level of phenolic compounds, leaf relative water content and water use efficiency, as well as, the variable chlorophyll fluorescence decreased during progressive drought.

4. The reduction was proportional to the intensity of the water stress and appeared to occur in a genotype-dependent manner.

Literature cited

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Acknowledgement

The present work was supported by the EU-funding grant POSCCE-A2-O2.1.2-2009-2 ID.524, cod SMIS-CSNR 11986.

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