Journal of Plant Physiology 202 (2016) 1–9

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Biochemistry

Different allocation of carbohydrates and phenolics in dehydrated leaves of triticale

Tomasz Hura[a,*], Michał Dziurka[a], Katarzyna Hura[b], Agnieszka Ostrowska[a], Kinga Dziurka[a]

[a] Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Kraków, Poland
[b] Department of Plant Physiology, Faculty of Agriculture and Economics, Agricultural University, Podłużna 3, 30-239 Kraków, Poland

ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 16 June 2016
Accepted 16 June 2016
Available online 15 July 2016

Keywords:
Drought stress
Soluble carbohydrates
Cell wall carbohydrates
Starch
Soluble phenolics
Cell wall-bound phenolics

ABSTRACT

Carbohydrates are used in plant growth processes, osmotic regulation and secondary metabolism. A study of the allocation of carbohydrates to a target set of metabolites during triticale acclimation to soil drought was performed. The study included a semi-dwarf cultivar 'Woltario' and a long-stemmed cultivar 'Moderato', differing in the activity of the photosynthetic apparatus under optimum growth conditions.

Differences were found in the quantitative and qualitative composition of individual carbohydrates and phenolic compounds, depending on the developmental stage and water availability. Soluble carbohydrates in the semi-dwarf 'Woltario' cv. under soil drought were utilized for synthesis of starch, soluble phenolic compounds and an accumulation of cell wall carbohydrates. In the typical 'Moderato' cv., soluble carbohydrates were primarily used for the synthesis of phenolic compounds that were then incorporated into cell wall structures. Increased content of cell wall-bound phenolics in 'Moderato' cv. improved the cell wall tightness and reduced the rate of leaf water loss. In 'Woltario' cv., the increase in cell osmotic potential due to an enhanced concentration of carbohydrates and proline was insufficient to slow down the rate of leaf water loss.

The mechanism of cell wall tightening in response to leaf desiccation may be the main key in the process of triticale acclimation to soil drought.

1. Introduction

The challenge of growing crops in areas frequently experiencing soil drought is the unpredictability of the scope and consequences of water shortage in plants (Boyer 1982). Therefore, breeding efforts have been implemented to obtain cultivars characterized by improved drought tolerance and water use efficiency (Araus et al., 2002). Studies determining key morphological, physiological, biochemical or molecular mechanisms of plant acclimation to drought are an important step in producing such varieties (Souza et al., 2004; Hura et al., 2007; Khanna-Chopra and Selote, 2007).

Currently, the data on changes in cell wall chemical composition during leaf dehydration are sparse. One of many hypotheses claims a role of cell wall-bound phenolics in plant acclimation to drought, as intensified incorporation of phenolic compounds into the cell wall may reduce carbohydrate utilization in the process of cell wall biomass growth (Kamisaka et al., 1990; Wakabayashi et al., 1997). Carbohydrates that are not incorporated into the cell wall increase cell osmotic potential, thus retaining water in the cell, maintaining appropriate turgor pressure and proper functioning of the photosynthetic apparatus (Hura et al., 2012). Saturation of the cell wall with phenolic compounds makes it less elastic and more dense and tight (Fry, 1979; Cushman, 2001). It also becomes more hydrophobic due to the presence of benzene rings, preventing water movement from the symplast into the apopolast and within the apoplast, and limits water transfer outside the cells (Kamisaka et al., 1990; Wakabayashi et al., 1997; Fry, 1982). Moreover, phenolic compounds incorporated into the cell wall serve as a filter, protecting the cells against high-energy UV radiation (Burchard et al., 2000). This is especially important during drought stress, which predisposes the photosynthetic apparatus under excess light to photoinhibitory injuries (García-Plazaola and Becerril, 2000; Bartels and Sunkars, 2005).

Carbohydrates are used in plant growth processes, osmotic regulation and secondary metabolism. Therefore, the aim of the

* Corresponding author at: Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Kraków, Poland.
E-mail address: t.hura@ifr-pan.edu.pl (T. Hura).

http://dx.doi.org/10.1016/j.jplph.2016.06.018
0176-1617/

study was to determine the allocation of resources to a target set of metabolites during triticale acclimation to soil drought stress applied at tillering, heading and the anthesis stage. The study included a semi-dwarf cultivar 'Woltario' and a long-stemmed cultivar 'Moderato'. Under optimum growth conditions, 'Woltario' cv. is characterized by higher activity of the photosynthetic apparatus than 'Moderato' cv. We assumed that differences in photosynthetic activity would be reflected in different manners of carbohydrate utilization during plant acclimation to drought. We performed quantitative analyses of starch, soluble carbohydrates and those released after cell wall hydrolysis, soluble phenolic compounds and cell wall-bound phenolics.

2. Materials and methods

2.1. Plant material

The study included two cultivars of winter triticale differing in their morphological traits, i.e. 'Moderato', a typical long-stemmed cultivar and a semi-dwarf 'Woltario' cultivar. Seeds were obtained from Danko Plant Breeders Ltd., Choryń, Poland.

2.2. Growth conditions and treatments

Seedlings were grown in plastic pots 3.7 dm^3 in volume, filled with a homogeneous mixture of soil and sand (1:3; v/v). At the stage of 1 leaf, the seedlings were subjected to vernalization for 8 weeks at +4 °C (±1 °C) with 10 h illumination and with a photosynthetic photon flux density (PPFD) of 200 $\mu mol\, m^{-2}\, s^{-1}$. After vernalization, the seedlings were transferred into an air-conditioned greenhouse chamber to a 16 h light/8 h dark photoperiod, a temperature of 23/18 °C (±2 °C) day/night, 40 ± 5% relative air humidity (RH) and a photosynthetic photon flux density (PPFD) of 250–350 $\mu mol\, m^{-2}\, s^{-1}$ at the level of the top leaf. Light intensity at the leaf level was measured with a QSPAR Quantum Sensor (Hansatech Instruments LTD, Kings Lynn, England). The plants were irrigated once a week with a full-strength Hoagland's solution (Hoagland, 1948; Hoagland and Arnon 1950).

In both cultivars, watering was restricted for three weeks at tillering, heading and anthesis, and for two weeks the soil water content in the drought stress variant was maintained at ca. 35% (75% in control). Soil water content was monitored gravimetrically, taking into account the weight of plants growing in the pots.

Measurements were performed on the 21st day of limited watering. The first top fully developed leaves were collected from drought-exposed plants analyzed at the tillering stage, whereas the analyses at heading and anthesis involved flag leaves.

2.3. Leaf water content

Leaf water content (LWC) was analyzed by quantitative sampling of leaf fresh mass (L_{FW}), followed by drying at 70 °C for 48 h, and weighing the resulting dry mass (L_{DW}). Water content was calculated according to the following equation and expressed as a percentage:

$LWC = ((L_{FW} - L_{DW})/L_{FW}) \times 100\%$. The measurements for each cultivar within treatments and growth periods were taken in 10 replicates (10 replicates means 10 plants).

2.4. Leaf osmotic potential

Measurements were performed with a psychrometer HR 33T (WESCOR, Inc., Logan, Utah, USA) equipped with leaf sample chambers C-52 (WESCOR). Leaf osmotic potential was evaluated for the sap squeezed out of leaf discs (Ø = 5 mm) with a syringe. Filter paper discs soaked in sap were placed in the chambers and left for 30 min. The measurements were taken in the dew point mode. The measurements for each cultivar within treatments and growth periods were taken in 5 replicates.

2.5. Soluble carbohydrates

Sugars were analyzed according to the method reported by Janeczko et al. (2013), with modifications. About 10 mg of lyophilized and homogenized samples was extracted in 1 ml of ultra-pure water (Option R, Elga, UK) by shaking for 15 min at 30 Hz (MM 400, Retsch, Germany). Then, the samples were centrifuged for 5 min at 21000 × *g* (Universal 32R, Hettich, Germany). Supernatant was collected, diluted with acetonitrile 1:1 (v/v), filtered (0.22 μm nylon membrane, Costar Spin-X, Corning, USA), and analyzed on HPLC for soluble sugar content. Pellet was kept for further starch and insoluble cell wall fractions analysis. The measurements for each cultivar within treatments and growth periods were taken in 5 replicates.

2.6. Starch

Starch was estimated as glucose released after enzymatic hydrolysis of pellets left after the soluble sugar analysis (Bach et al., 2015). Alpha-amylase in 50 mM potassium phosphate buffer pH 6.9 with 6.7 mM of NaCl, and amyloglucosidase in 200 mM sodium acetate buffer pH 4.5 were used. The pellets were rinsed twice with 1 ml of ultrapure water and centrifuged each time. Water was decanted and 350 μl of α-amylase solution (8 μg of lyophilized enzyme per 1 ml of phosphate buffer) were added, and then the samples were vortexed and placed in boiling water bath for 10 min. Next, the samples were cooled down and 450 μl of amyloglucosidase were added (35 μg of lyophilized enzyme per 1 ml of acetate buffer), and the samples were placed in a 50 °C water bath for one hour. Then, they were centrifuged, supernatant was collected, diluted with acetonitrile 1:1 (v/v) and analyzed by HPLC for glucose content. Pellets were used for insoluble plant cell wall components (cellulose, arabinoxylans) analysis. The measurements for each cultivar within treatments and growth periods were taken in 5 replicates.

2.7. Cell wall carbohydrates

Cell wall components were analyzed according to Uddin et al. (2014), with modifications. Hydrolysis of cellulose and the water insoluble fraction of arabinoxylans was achieved by an initial dispersion in 72% (w/w) sulfuric acid followed by hydrolysis in 0.25 M sulfuric acid. After the sample pellets were rinsed with water, 200 μl of 72% sulfuric acid was added. The samples were left for 1 h at 30 °C, and after that the suspension was diluted with 5.6 ml of ultrapure water and incubated for 1.5 h at 120 °C. The hydrolyzate was neutralized with saturated NaOH, filtered and analyzed by HPLC. The measurements for each cultivar within treatments and growth periods were taken in 5 replicates.

2.8. HPLC separation of neutral sugars

High performance liquid chromatography analyses of free sugars, starch and cell wall components hydrolyzate were performed using an Agilent 1200 chromatograph equipped with a degasser, binary pump, automated liquid sampler and thermostated column compartment (Agilent, Germany) and ESA Coulochem II electrochemical detector with 5040 Analytical Cell (ESA, USA) with