Journal of Plant Physiology 169 (2012) 686–695

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Responses of growth and primary metabolism of water-stressed barley roots to rehydration

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ARTICLE INFO

Article history:
Received 6 September 2011
Received in revised form 9 January 2012
Accepted 10 January 2012

Keywords:
Drought stress
Hordeum vulgare
Metabolite analysis
Nitrogen metabolism
Osmotic adjustment
Water deficit

ABSTRACT

Barley seedlings were grown in pots in controlled environment chambers and progressive drought treatments were imposed 11 d after sowing. Soil water content decreased from 92 to 10% following 14 d without watering. Increases of biomass in shoots and roots slowed after 4 and 9 d of water stress, respectively. Thirty barley root metabolites were monitored in this study and 85% were significantly altered by drought. Sucrose, raffinose, glucose, fructose, maltose, malate, asparagine and proline increased and myo-inositol, glycerate, alanine, serine, glycine and glutamate decreased during drought. Primary metabolism was likely involved in various crucial processes during water stress including, osmotic adjustment, nitrogen sequestration and ammonia detoxification. Rates of photosynthesis and stomatal conductance recovered in 2 d and shoot growth commenced the 3rd day after rehydration. Root growth also exhibited a lag after rehydration but this was attributed to high nutrient concentrations during water stress. Malate and proline recovered within 1 d but serine was only partially reversed 6 d after rehydration. Malate, aspartate and raffinose decreased below well-watered, control levels following rehydration. Variation in the magnitude and time necessary for individual compounds to fully recover after rehydration suggested the complexity of metabolic processes initiated by re-watering.

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Introduction

Drought is a common cause of crop failure due to inadequate rainfall and to seasonal fluctuations of soil moisture. The diversion of fresh water from agriculture to other purposes and changing climate due to a buildup of greenhouse gasses in the atmosphere are predicted to worsen episodes of drought, particularly in growing regions that are already prone to water deficits (IPCC, 2007). Consequently, enhancing the drought tolerance of major crop species is an important goal of modern plant breeding (Boyer, 1982). Barley is moderately drought tolerant and is a preferred crop in cool, semi-arid growing regions (Poehlman, 1985). Enhancing the stress tolerance of barley and other important crop species will be needed to cope with predicted increases in the frequency and severity of drought.

Plants possess a variety of adaptive mechanisms to withstand desiccation. Two widely recognized responses of plants to low SWC are stomatal closure and an increase in the root/shoot ratio. Drought dependent stomatal closure occurs rapidly in response to soil moisture deficits and this negatively impacts rates of CO_2 assimilation. Shoot growth is generally more susceptible to soil water deficits than root growth and the ability of roots to proliferate in water deficient soils allows plants to survive when moisture is limiting (Pace et al., 1999; Sharp et al., 2004). Water stress also disrupts metabolic processes involved in CO_2 assimilation and the combined effects of stomatal closure and metabolic impairment ultimately decrease rates of biomass accumulation (Flexas et al., 2004). Turgor is typically maintained during water stress by osmotic adjustment and this requires the synthesis and transport of compatible solutes and functionally important osmolytes, such as soluble sugars, polyols, Pro and Gly betaine (Foyer et al., 1998; Morison et al., 2008). Sharp et al. (2004) proposed that hexoses and Pro were responsible for one half of the osmotic adjustment in maize root tips in response to water deficiency. Compounds involved in primary metabolism also are important precursors of stress related compounds, such as phenylpropanoids, flavonoids and proteins (Dixon and Pavia, 1995). A second role of primary metabolites during plant stress is the mitigation of oxidative stress (Shen et al., 1997; Taji et al., 2002) due to the formation of reactive oxygen species in roots in response to desiccation (Roach and Kranner, 2011). Water deficits decrease the uptake of nutrients from the soil and impede the distribution of nutrients within

Abbreviations: A, net rate of CO_2 assimilation; C_i, intercellular CO_2 concentration; DAS, days after sowing; DW, dry weight; FW, fresh weight; g_s, stomatal conductance; LWP, total leaf water potential; PPFD, photosynthetic photon flux density; SWC, soil water content; SWP, total soil water potential.

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0176-1617/$ – see front matter. Published by Elsevier GmbH.
doi:10.1016/j.jplph.2012.01.002

the plant via the transpiration stream (Taylor et al., 1982). Consequently, nutritional imbalances have been observed in plants subjected to water stress (Heckathorn et al., 1997). Collectively, rapid adjustments of primary plant metabolism are important in the adaptive responses of plants to water stress and understanding these metabolic processes could lead to enhanced plant performance under stressed conditions.

Roots are in direct contact with the soil and are the earliest organ on the plant to sense water deprivation. The length, volume and cell wall chemistry of roots are modified during prolonged water stress (Nobel and Huang, 1992; Sharp et al., 2004; Ober and Sharp, 2007). Chemical signals, including ABA and cytokinins, originate in the root, are transferred to the shoot via the xylem and contribute to the regulation of stomatal closure and the inhibition of shoot growth in response to water deficiency (Schachtman and Goodger, 2008).

The goal of the current study was to better understand changes of primary metabolism in barley roots due to water stress and during subsequent rehydration. The reversal of chemical and morphological changes in roots due to desiccation is crucial to plant survival and crop productivity. It was previously reported (Taylor et al., 1982; Prasad et al., 1982) that a limited number of stress-related root metabolites reverted to control levels 3 d after desiccated plants were re-watered. Effects of rehydration on a broader array of root metabolites have not been examined in detail. Also, it is not clear if various metabolic pathways in roots recover from the effects of water stress at the same rate. Differences in the time required for a metabolic pathway to respond to re-watering may indicate a pathway's relative importance in the recovery process. Stress dependent changes of primary metabolism have been studied more extensively in shoots than roots and additional information on drought processes affecting root metabolism could be insightful.

Materials and methods

Plant growth

Barley seedlings (*Hordeum vulgare* L. cv. Brant) were grown in a matching pair of controlled environment chambers (Model M-2, EGC Corp., Chagrin Falls, OH, USA) providing a 14 h photoperiod and a 24 h day/night cycle as described previously (Sicher and Bunce, 2008). Plants were seeded in 1 dm^3 plastic pots filled with vermiculite, the air temperature was $22 \pm 1\,^{\circ}C$, the CO_2 partial pressure was maintained at 38 ± 1 Pa, and the PPFD was $550 \pm 40\ \mu mol\ m^{-2}\ s^{-1}$ when measured at pot height. Pots were thinned to single plants 7 DAS and relative humidity in both growth chambers was between 60 and 80% when measured during the light period. Plants were watered daily with a complete mineral nutrient solution containing 12.5 mM inorganic nitrate and 2.5 mM ammonium. Water stress treatments were initiated 11 DAS by withholding nutrient solution from all of the seedlings in one of the two controlled environment chambers. Drought treatments were maintained for a total of 14 d. However, nutrient solution was re-administered to one-half of the water depleted plants following 8 d of water stress treatment and this watering regime was continued until experiments were terminated 6 d later. All of the plants that were rehydrated following 14 d of water stress survived the drought treatment. Roots were harvested at indicated times and were quickly washed to remove any associated vermiculite. The washed roots were blotted dry with paper towels and placed in labeled paper bags. All root samples were immediately transferred to liquid N_2 and lyophilized. The freeze dried root tissue was then stored at $-80\,^{\circ}C$ for up to two months prior to use.

Metabolite analysis

Individual barley roots were ground to a fine powder under liquid N_2 in a mortar and pestle and approximately 25 mg of the pulverized root tissue was extracted at $4\,^{\circ}C$ with 1.4 mL of ice-cold methanol in a ground glass tissue homogenizer. The homogenates were collected in 15 mL Falcon tubes, incubated at $70\,^{\circ}C$ in a H_2O bath for 15 min and diluted with an equal volume of deionized H_2O. After centrifugation for 15 min at $5800 \times g$ in a Beckman Coulter centrifuge (model Avanti J-20 XP, Palo Alto, CA, USA) a 25 μL aliquot of the supernatant was placed in a 1 mL reactivial and dried overnight under vacuum. Organic acids and soluble carbohydrates were subsequently quantified by the metabolite profiling technique described by Roessner et al. (2000). Details of the chromatographic separation, detection and quantitation of these compounds have been described earlier (Sicher, 2008). Pellets from the centrifugation step were washed three times with 1 mL of methanol, dried overnight and then used for the determination of starch as previously described (Sicher, 2008). Internal standards containing 62.5 nmol of α-aminobutyric acid and 26 μmol of ribitol were injected into each sample prior to homogenization.

A 1 mL aliquot of the aqueous–methanol extract was used for the measurement of soluble amino acids by the AccQ-Tag Ultra procedure according to the manufacturer's instructions (Waters Corp., Milford, MA). Samples were evaporated to dryness, resuspended in 0.1 mL of 20 mM HCl and filtered by microcentrifugation using 0.22 μm Ultrafree-MC filter units (Millipore Corp, Billerica, MA, USA). Amino acid separations were performed at $55\,^{\circ}C$ on a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system equipped with a 2.1×100 mm AccQ-Tag Ultra column for amino acid analysis as described by the manufacturer. Individual peaks were detected by absorbance with an Acquity TUV detector set to 260 nm. The output of the detector was monitored at a sampling rate of 10 datapoints s^{-1} using Empower2 Plus software from the instrument manufacturer. Standard curves were prepared with a known mixture of 19 amino acids for each sample set. Except for Glu and Arg the amino acid standards, plus ammonia and the internal standard were completely separated by using these methods.

Gas exchange measurements

Gas exchange parameters were usually measured between 3 and 6 h after the start of the light period using individual barley leaves just prior to sampling for metabolite analyses. Measurements were initiated on the 8th d of water stress treatment and were continued at 1 or 2 d intervals. Net CO_2 and H_2O vapor exchange rates were determined with a CIRAS-2 photosynthesis system (PP systems, Amesbury, MA, USA) using a broadleaf chamber with a 2.5 cm^2 window. Individual leaves from a single barley seedling were placed in the cuvette with conditions set to match that of the growth chambers used for plant growth. A PPFD of $550 \pm 20\ \mu mol\ m^{-2}\ s^{-1}$ was obtained using the red/blue LED source provided with the instrument. Leaf temperature was $22 \pm 0.2\,^{\circ}C$, the CO_2 partial pressure was 38 Pa and relative humidity was 60%. Leakage rates during gas-exchange measurements were determined with an empty cuvette. Gas exchange data were collected using three to five plants from the water sufficient and insufficient treatments on indicated dates. Values of A, C_i and g_s were calculated by the Photosynthesis System. Immediately after completing the gas-exchange analyses leaf discs were removed from the measured leaves and leaf water potential was determined with a model HR-33T dewpoint microvoltmeter (Wescor, Logan UT, USA).

Other methods

For biomass determinations plants were separated into root and shoot fractions and these were dried in a forced air oven at $70\,^{\circ}C$