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Effects of CO_2 enrichment and drought pretreatment on metabolite responses to water stress and subsequent rehydration using potato tubers from plants grown in sunlit chambers

Jinyoung Yang[a,*], David H. Fleisher[a], Richard C. Sicher[a], Joonyup Kim[b], Virupax C. Baligar[c], Vangimalla R. Reddy[a]

[a] U.S.D.A., Agricultural Research Service, Crop Systems and Global Change Laboratory, Room 342, Bldg. 001, Beltsville Agricultural Research Center-west, 10300 Baltimore Avenue, Beltsville, MD 20705, USA
[b] U.S.D.A., Agricultural Research Service, Soybean Genomics and Improvement Laboratory, Room 207, Bldg. 006, Beltsville Agricultural Research Center-west, 10300 Baltimore Avenue, Beltsville 20705 MD, USA
[c] U.S.D.A., Agricultural Research Service, Sustainable Perennial Crops Laboratory, Room 223, Bldg. 001, Beltsville Agricultural Research Center-west, 10300 Baltimore Avenue, Beltsville, 20705 MD, USA

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ABSTRACT

Experiments were performed using naturally sunlit Soil–Plant–Atmosphere Research chambers that provided ambient or twice ambient CO_2. Potato plants were grown in pots that were water sufficient (W), water insufficient for 12–18 days during both vegetative and tuber development stages (VR), or water insufficient solely during tuber development (R). In the ambient CO_2 treatment, a total of 17 and 20 out of 31 tuber metabolites differed when comparing the W to the R and VR treatments, respectively. Hexoses, raffinose, mannitol, branched chain amino acids, phenylalanine and proline increased, although most organic acids remained unchanged or decreased in response to drought. Osmolytes, including glucose, branched chain amino acids and proline, remained elevated following 2 weeks of rehydration in both the ambient and elevated CO_2 treatments, whereas fructose, raffinose, mannitol and some organic acids reverted to control levels. Failure of desiccated plant tissues to mobilize specific osmolytes after rehydration was unexpected and was likely because tubers function as terminal sinks. Tuber metabolite responses to single or double drought treatments were similar under the same CO_2 levels but important differences were noted when CO_2 level was varied. We also found that metabolite changes to water insufficiency and/or CO_2 enrichment were very distinct between sink and source tissues, and total metabolite changes to stress were generally greater in leaflets than tubers.

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1. Introduction

Potato is the foremost root crop cultivated globally and its importance derives from the production of abundant starch filled tubers. The potato plant is cultivated as an herbaceous annual that transfers assimilates from leaves to underground stolons, where tuber formation occurs (King and Stark, 1997). Potato performs well in varying climates but optimal tuber yields occur when plants are grown in cooler regions with abundant soil moisture. With advanced farming techniques, potato yields can exceed 40 Mt ha^{-1} (Ojala et al., 1990). The maximal soil temperature for tuber formation is approximately 25 °C (Menzel, 1985) but yield potential may diminish as future climates become warmer and drier (Evers et al., 2010). Potato is shallow rooted and tuber yield is particularly susceptible to soil water deficits (King and Stark, 1997; Ojala et al., 1990). Potato tubers that are grown with water deficits are often unsuitable for processing and sale, partly because of decreased starch and elevated sugar levels (Ojala et al., 1990; Kumar et al., 2004). This condition contributes to browning and/or acrylamide formation at high temperatures used for cooking (Ohara-Takada et al., 2005). Therefore, adequate hydration, especially during key developmental stages, is essential for producing and marketing potato tubers.

Abbreviations: Amb, ambient; Elv, elevated; R, reproductive drought treatment; VR, vegetative and reproductive drought treatment; W, well-watered treatment; Deh, dehydrated treatment; Reh, rehydrated treatment; SWC, soil water content.

* Corresponding author. Fax: +1 301 504 5823.
E-mail address: jinyoung.barnaby@ars.usda.gov (J. Yang).

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Because potato is a C_3 crop, significant yield increases occur in response to CO_2 enrichment (Kimball, 1983). A 40% increase in tuber yields was observed when CO_2 concentrations were increased from 460 to 660 μmol mol^{-1} CO_2 using a Free Air CO_2 Enrichment technique (Miglietta et al., 1998). A similar increase in tuber yields was observed when experiments were performed in open-topped chambers in the field (Sicher and Bunce, 1999). Both of these prior studies observed increased tuber number in response to CO_2 enrichment. Therefore, it is possible that large below ground tubers function as sinks for excess assimilates formed by exposure to Elv CO_2. Although potato leaflets accumulate high starch levels in response to Elv CO_2 treatments (Sicher and Bunce, 2001), tuber development can partially relieve the feedback inhibition of net photosynthesis that is attributed to leaf starch (Ludewig et al., 1998; Sicher and Bunce, 1999). Stomatal aperture also is decreased by Elv CO_2 concentrations in the atmosphere and this can result in enhanced water use efficiencies for many C_3 crops (Bunce, 2004; Barnaby and Ziska, 2012). However, the effects of CO_2 enrichment on plant water relations generally are greater for mild rather than severe drought conditions (Sicher and Barnaby, 2012).

Fleisher et al. (2013, 2014) recently reported results of experiments on potato plants grown in Soil–Plant–Atmosphere–Research chambers that were exposed to cyclic drought treatments under ambient or elevated CO_2. One important observation was that drought treatments increased the harvest index of potato. This was consistent with data from other species showing that shoot growth was retarded and root growth was promoted by water deficits (Sicher et al., 2012). Several prior authors (Bussis and Heineke, 1998; Vasquez-Robinet et al., 2008; Evers et al., 2010; Kondrak et al., 2012; Barnaby et al., 2015) reported metabolite changes in drought treated potato leaflets or potato tubers (Muttucumaru et al., 2015; Juhász et al., 2014; Maggio et al., 2008). In general, reducing sugars and specific amino acids, including Pro, accumulated, whereas starch and certain organic acids decreased in response to drought stress. These prior studies confirmed that low molecular weight metabolites were integrally involved in abiotic stress mitigation in plants (Kaplan et al., 2004; Pinheiro et al., 2004; Sperdouli and Moustakas, 2012; Pedrol et al., 2000). The above mentioned metabolite studies on potato were all single treatment experiments. However, it is unclear how drought pre-treatments, CO_2 enrichment or the reversal of water stress by rehydration affected these results. This detailed information is important if we are to understand the effects of complex future environments on potato production. In addition, no one has compared stress dependent changes of tuber and leaf metabolites from the same experiment. It is highly likely that the extent of drought stress differs for below and above ground tissues and this should affect metabolite responses to water stress.

Greenhouse gas concentrations in the atmosphere are increasing and current predictions are that this will result in rising temperatures and altered precipitation patterns (Cao et al., 2011). The objective of this paper was to determine metabolite responses to CO_2 enrichment and water insufficiency in potato tubers and to compare this to foliar data from the same experiment. Because climate change should also increase the frequency and duration of drought, we questioned whether previous episodes of water stress affected metabolite responses to subsequent water stress treatments. To accomplish this, drought treatments were imposed solely during reproductive growth (R) or during both vegetative and reproductive growth (VR) combined. Tuber samples were also collected on the final day of the treatment (Deh, dehydration samples) and 2 weeks of after watering was restored (Reh, rehydrated samples). We further postulated that elevated CO_2 treatments would preserve soil moisture and mitigate the effects of water depletion on potato tubers.

2. Materials and methods

2.1. Plant materials

Two consecutive plantings were performed during the 2011 growing season using six naturally sunlit Soil–Plant–Atmosphere–Research chambers at the Beltsville Agricultural Research Center, in Beltsville, Maryland, U.S.A. Air temperature, chamber air CO_2 concentrations, irrigation, nutrient supply and humidity were controlled as described previously (Fleisher et al., 2013; Barnaby et al., 2015). Twelve, 16 l pots per chamber were filled with a 3:1 ratio of washed sand and vermiculite (Grace Construction Products, Cambridge, MA., USA). Sections of seed tubers (*Solanaum tuberosum* L. var. Kennebec), that averaged 50 g FW, were sown in each pot. Sprouts were thinned to two main stems per pot between 5 and 6 days after emergence. Chamber air was maintained at either 400 (Amb) or 800 (Elv) μmol mol^{-1} CO_2 during daylight and was uncontrolled at night. Air temperatures were 22 ± 0.3 °C during the day and 17 ± 0.3 °C during the night. The relative humidity varied between 62 and 78%. The average daily integral of photosynthetically active radiation was 43.9 ± 10.1 and 24.7 ± 12.4 mol m^{-2} in the first and second plantings, respectively. First, reproductive drought treatments (R) commenced between 42 and 45 days after shoot emergence using one SPAR chamber from each CO_2 treatment. Water was withheld for 12–18 days and then all pots were rehydrated when leaf water potential (LWP) was −1.5 MPa or less (Barnaby et al., 2015). A second pair of SPAR chambers was pre-treated with drought during the vegetative growth phase prior to receiving the above reproductive drought treatments (VR). For the vegetative drought pre-treatment, water was withheld beginning 10 days post emergence. Watering was resumed approximately 14 days later based on changes of leaf water potential as described above. The final two SPAR chambers served as well-watered controls (W). Leaf water potential was measured with a model HR-33T dewpoint microvoltmeter after a 1 h incubation period (Wescor, Logan UT, USA). The volumetric soil water content of the potting medium was determined by time domained reflectrometry and data were collected hourly using seven pots in each SPAR chamber (Fleisher 2013).

2.2. Metabolite measurements

A random tuber was harvested from four separate, individual pots in each of the six SPAR chambers on the last day of drought treatment of both plantings. A second random tuber from four additional pots in each chamber was harvested 14 days later, when all pots were fully rehydrated. The selected tuber from each pot was removed from the soil medium, quickly rinsed and a 1 cm diameter core of tissue was removed from the center of the tuber with a cork borer. The periderm of each core sample was removed with a sharp razor and the remaining medulla tissue was transferred to labeled envelopes and immediately frozen in liquid N_2. Samples were then freeze–dried and stored at −20 °C for up to two months. Lyophilized tuber tissue was ground to a fine powder in a mortar and pestle and 30 mg dry weight was pulverized in a TissueLyser II bead mill (Qiagen, Valencia, CA, USA) using 2 ml SealRite microcentrifuge tubes (USA Scientific, Ocala, FL USA) containing a 6.4 mm diameter ceramic bead (MP Biomedicals, Santa Ana, CA USA). Soluble metabolites were extracted in aqueous methanol and were quantified by gas chromatography coupled to mass spectrometry as described previously (Sicher and Barnaby, 2012; Yang et al., 2014; Barnaby et al., 2015). Standard curves were prepared with four point curves using known concentrations of soluble sugars, sugar alcohols, organic acids and amines.