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Research article

Effects of growth temperature and carbon dioxide enrichment on soybean seed components at different stages of development

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ABSTRACT

Soybean plants were grown to maturity in controlled environment chambers and at the onset of flowering three temperature treatments were imposed that provided optimum [28/24 °C], low [22/18 °C] or high [36/32 °C] chamber air temperatures. In addition, plants were treated continuously with either 400 or 800 μmol mol^{-1} CO_2. Seeds were harvested at 42, 53, 69 and 95 days after planting (i.e., final maturity). This study quantified 51 metabolites in developing soybean seeds, plus total lipids and proteins were measured at maturity. About 80% of measured soluble carbohydrates, amines and organic acids decreased to low levels in mature seeds, although important exceptions were raffinose, ribose/arabinose, citrate and all eight fatty acids. This suggested that the metabolism of young seeds supported lipid and protein synthesis. A total of 35 and 9 metabolites differed among temperature and CO_2 treatments, respectively, and treatment effects were predominately observed on the first and second samplings. However, shikimate, pinitol and oleate were increased by high temperature treatments in mature seeds. The above results indicated that CO_2 enrichment primarily altered metabolite levels during the initial stages of seed development and this was likely due to enhanced photosynthate formation in leaves.

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1. Introduction

A changing climate increases the possibility of abiotic stress events that can diminish crop yields and negatively affect seed quality (Prasad et al., 2005). Global temperatures are predicted to increase 1.5–4.5 °C above current values by the end of 21st century and both plant development and seed quality can be negatively impacted by both sub- (low, LT) or by supra-optimal (high, HT) growth temperatures (Öquist, 1983; Porter, 2005). Seed growth rate and soybean yields were diminished when air temperatures exceeded 29–33 °C (Egli and Wardlaw, 1980; Dornbos and Mullen, 1992; Puteh et al., 2013) and the effects of temperature stress were greater during reproductive compared to vegetative plant growth (Baker et al., 1989). The influence of air temperature on soybean seed growth varies depending on the stage of development and on the length of temperature stress treatment. Generally, increasing the duration of temperature stress during the middle period of reproductive growth was more harmful for seed yield and seed size than were shorter periods of temperature stress during either early or late seed growth (Egli and Wardlaw, 1980; Puteh et al., 2013).

Atmospheric CO_2 concentrations may double by the end of this century from a current level of approximately 400 μmol mol^{-1} (Shiogama et al., 2016). Elevated CO_2 (eCO_2) enhances photosynthetic rates of C_3 plants, such as soybean, and this normally results in increased seed yield (Baker et al., 1989; Allen et al., 1998). However, observed yield enhancements in response to eCO_2 can be

Abbreviations: aCO_2, ambient CO_2; Ala, alanine; Arg, arginine; Asn, asparagines; Asp, aspartic acid; Cys, cysteine; eCO_2, elevated CO_2; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; HT, high temperature; Ile, isoleucine; Leu, leucine; LT, low temperature; Lys, lysine; Met, methionine; OT, optimum temperature; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Try, tryptophan; Tyr, tyrosine; Val, valine.

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negatively impacted by high temperature stress (Thomas et al., 2003; Allen et al., 1998). It is widely agreed that the negative effects of enhanced growth temperatures on the yield and quality of mature grain produced by legumes is not mitigated by eCO_2 treatments (Gibson and Mullen, 1996; Piper and Boote, 1999; Prasad et al., 2005). However, the impact of CO_2 enrichment on seed components during early development has received almost no attention.

Soybean seeds are a major source of high value nutritional compounds and it has been estimated that soybean provides about 20% of the protein and 25% of the lipids necessary for both human consumption and animal feed (Prasad et al., 2005). Soybean seeds typically contain about 30% carbohydrate, 30–40% protein and 19% lipid and are a vital dietary source of essential fatty acids and amino acids (Hymowitz et al., 1972; Zarkadas et al., 1999; Medic et al., 2014). Soybean seed components are derived from the translocation of compounds formed by photosynthetic reactions in leaves. The primary photosynthetic product used in soybean seed development is sucrose (Fader and Koller, 1985), which is catabolized in immature seeds to phosphoenol pyruvate (PEP), a key metabolic intermediate in both protein and lipid synthesis. Enzymes related to PEP metabolism, such as PEP carboxylase and pyruvate kinase, regulate the amount of carbon partitioned into protein and lipid during seed development (Smith et al., 1989).

Seed composition is genetically controlled and also is affected by key environmental factors (Baker et al., 1989; Rotundo and Westgate, 2009). Enhancing growth temperatures above a 26–29 °C temperature threshold simultaneously increased seed protein and decreased lipid content (Dornbos and Mullen, 1992; Gibson and Mullen, 1996; Rotundo and Westgate, 2009). However, both protein and lipid levels decreased sharply when the air temperature exceeded 40 °C during reproductive plant development (Thomas et al., 2003). Enhancing growth temperatures below a 25–26 °C temperature threshold increased lipid content but total seed protein was unaffected. With the exception of Met, amino acids from protein hydrolysates were unchanged by varying growth temperature in mature soybean seeds (Wolf et al., 1982). In general, unsaturated fatty acids are produced in greater quantities when plants are grown at low temperature (LT) than at HT. The essential fatty acids, linolenic (18:3) and linoleic (18:2) acid, decrease under HT treatments and there is a commensurate increase in palmitic (18:0) and oleic (18:1) acid (Wolf et al., 1982). Concentrations of organic acids are relatively low in soybean seeds and these compounds are thought to be little affected by varying growth temperatures during seed development (Yazdi-Samadi et al., 1977).

Soluble components of mature soybean seeds also can be altered by growth temperature, whereas the effects of CO_2 enrichment are thought to be negligible (Prasad et al., 2005). For example, total non-structural carbohydrates (TNC) decreased in soybean seeds in response to high temperature (HT) treatments and acute HT treatments had the greatest effects on seed composition. Moreover, glucose, fructose and raffinose were unchanged but sucrose content decreased significantly in soybean seeds as the growth temperature was increased from 18/13 °C to 33/28 °C (Wolf et al., 1982). Various authors (Baker et al., 1989; Allen et al., 1998; Thomas et al., 2003) observed that soluble sugar levels in mature soybean seeds were unaffected by plant growth under eCO_2. Because eCO_2 treatments enhanced crop yields, it was likely this was due to increased seed number rather than to increased seed size. According to one study (Vu et al., 2001), night temperature was an important factor in determining the sugar content of mature seeds and it was postulated that HT at night stimulated carbohydrate utilization.

The effects of high temperature stress on the seed metabolome and proteome of soybean have been described previously. Recently, a total of 275 metabolites were identified in mature soybean seeds and the authors argued that compounds with antioxidant properties, such as flavonoids and phenylpropanoids, were enriched in the HT compared to optimum temperature (OT) treatments (Chebrolu et al., 2016). This was a clear indication that the regulation of oxidative stress is an important factor in heat stress tolerance during soybean seed development. Also, a shift from soluble carbohydrates to polyols occurs when soybean tissues are exposed to heat stress (Sicher, 2013). In addition, twenty polypeptides were identified in mature soybean seeds that were altered by HT treatments and these included seed storage proteins and a LEA protein (Ren et al., 2009).

Considering the economic importance of soybeans, numerous studies on soybean seed constituents exist and the interactive effects of temperature and eCO_2 on soybean seed components have been studied previously. However, prior investigations almost exclusively used mature seeds (Prasad et al., 2005) and much of this material was obtained piecemeal and used methods that lack the sensitivity and accuracy of modern instrumentation. We propose that a careful study of temperature by CO_2 interactions on a complex variety of constituents found in developing soybean seeds could provide important insights into processes affecting seed maturation and seed quality. Other than soluble carbohydrates, very little is known about primary metabolism in developing soybean seeds. In the current study, we hypothesized that eCO_2 is capable of mitigating temperature stress dependent changes of soybean seed composition and that the effects of eCO_2 treatments would be greater during the early compared to the final stages of seed development.

2. Materials and methods

2.1. Plant materials

Experiments were conducted at the USDA-ARS, Henry A. Wallace Agricultural Research Center in Beltsville, MD, USA. Soybean [*Glycine* max (L.) Merr., cv. NC-Roy] plants were grown from seed in six matching controlled environment chambers (EGC Corp., Chagrin Falls, OH, USA), essentially as described previously (Sicher, 2013). Replicate experiments were initiated in February and June of 2014. Seeds were sown in 7.6 L pots filled with a 50:50 mixture of pre-wetted vermiculite and Metro-Mix® and 16 individual pots were placed in each chamber. All chambers were initially set to provide 28/24 °C (OT), 12 h day/12 h light and 400 μmol mol^{-1} CO_2 and plants were watered with a complete nutrient solution using automatic drip irrigation. Pots were thinned to individual plants 7 days after planting (DAP). Beginning at the onset of flowering (approximately 25 DAP), two growth chambers were reprogrammed to provide 22/18 °C and two additional chambers were set to 36/32 °C. The remaining two of the six original chambers remained at the original temperature setting (28/24 °C). Simultaneously, one of the chambers in each of the three temperature treatments was switched from aCO_2 to eCO_2 (i.e., from 400 to 800 μmol mol^{-1} CO_2). Chamber conditions were randomly assigned and chamber settings were applied until plants were harvested at final maturity (95 DAP). Plants were irrigated 3 to 4 times a day with a complete, full strength Hoagland's nutrient solution until the drip point. Irradiance was 900 μmol m^{-2} s^{-1} (photosynthetically active radiation, PAR) and was provided by a combination of metal halide and high pressure sodium lamps. The relative humidity was not controlled but varied between 40% and 60%. Four pots from each chamber were harvested at 26, 42, and 53 DAP and the final harvest was performed 95 DAP at final maturity.