Nitrogen fixation and allocation of lentils subjected to heat stress under higher atmospheric CO_2 concentration

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Abstract

The rise in atmospheric CO_2 concentration from 400 to 550 ppm by 2050 will stimulate crop growth and yield and legumes may have a competitive advantage over non-legumes due to stimulation of N_2 fixation under elevated CO_2. However, heat stress is a major limitation to crop yield and elevated CO_2 may mitigate the effects of heat shock impacts. This study evaluated whether N_2 fixation and seed N yield of two lentil genotypes (PBA Ace and 05H010L-07HS3010, shortened HS3010 from here onwards) are buffered by heat waves impacts when grown under e[CO_2]. Lentils were grown under ambient CO_2 (a[CO_2], ~400 ppm) and or elevated CO_2 (e[CO_2], ~ 550 ppm) in the Australian Grains Free Air CO_2 Enrichment facility at Horsham, Victoria in 2015. A heat shock (40ºC) was imposed at flat pod stage for three consecutive days using custom built heat chambers. Under e[CO_2] symbiotically fixed N and total N content of both cultivars were significantly higher (11%) compared to a[CO_2]. Heat stress reduced N_2 fixation (19%) compared with the ambient temperature controls (CO_2 pooled). More soil N was taken up in heat stress than control treatments partly offsetting lower N_2 fixation. The decrease of seed N concentration by heat was more apparent under a[CO_2]. Seed N content and seed yield increased under e[CO_2] and the cultivar PBA had higher seed N and yield than HS3010. Heat stress reduced the seed N and yield of lentils more under a[CO_2] than e[CO_2]. Evidence from this study suggested that e[CO_2] may mitigate the deleterious effect of heat stress on N_2 fixation and yield of legumes.

Keywords

Elevated CO_2, N_2 fixation, N content, *Lens culinaris* L.

Introduction

Atmospheric CO_2 concentration ([CO_2]) has increased from 280 ppm before the Industrial Revolution to the current level of 400 ppm in 2015 and is expected to rise to 550 ppm by 2050 (IPCC 2013). This extremely rapid rise in [CO_2] causes critical uncertainty about climate change and its impact on food production systems. Net photosynthesis of C_3 plants is increased under e[CO_2], increasing carbohydrate acquisition and consequently, leading to better growth and yield (Ainsworth and Rogers 2007). Legumes may be more responsive to e[CO_2] than non-legumes due to stimulation of N_2 fixation (Rogers et al. 2009). However, N_2 fixation is also very sensitive to environmental stresses such as drought, salinity and heat stress (Aranjuelo et al. 2014).

Rising [CO_2] leads to increasing temperatures and increased frequency and severity of drought and heat waves are expected in many cropping areas (IPCC, 2013). Evidence suggests that e[CO_2] stimulates crop growth and alleviates heat stress for many C3 species (Yu et al. 2012; Fitzgerald et al. 2016). Most heat studies reported effects on above ground plant parts and only little attention has been paid on below ground parts, such as nodules and their N_2 fixation activities (Aranjuelo et al. 2014). Higher temperature during reproductive stages shortens the seed filling period, reduces translocation of assimilates (C and N) to developing seed and reduces seed yield (Dias and Lidon 2009). Yield reduction by heat stress has been reported for many crops including legumes such as soybean, pea, chickpea, and lentils (Kaushal et al. 2016). Heat waves (>35ºC for 6 days) in 2009 reduced lentil yield in Southern Australia by more than 70% (Nuttall et al. 2012). The combined effect of heat stress and e[CO2] on lentil N2 fixation and seed N yield are unclear. Additional C and stimulated N2 fixation under e[CO2] may buffer heat stress in legumes so that greater translocation rates of N to developing seeds during the seed filling period can be maintained. Therefore, we hypothesised that e[CO_2] will increase N2 fixation in lentil and will offset the adverse effect of heat waves on total N yield and seed quality of lentils.

Methods

The experiment was carried out at the Australian Grains Free Air CO2 Enrichment (AGFACE) facility Horsham, Victoria, Australia (36°45′70″S, 142°06′52″E, 127 m above sea level). Four FACE plots (or rings) with elevated [CO2] at ~550 ppm and four control plots (no rings) with ambient [CO_2] at ~400 ppm were used in this experiment. Two lentil cultivars (*Lens culinaris* L.) cv. PBA ACE and HS3010 (05H010L-07HS3010) were used in this experiment. Inoculated seeds (Group F® Rhizobium leguminosarum) were hand sown on 22 May, 2015. Plants were sown at a density of 200 plants m^{-2} and row spacing was 24.4 cm. The experiment was designed as a split–plot with CO_2 as the main plot, two lentil cultivars and heat treatments (control and heat) as sub-plot. Plants were subjected to heat waves at flat pod stage (137 days after sowing) for 3 days (6 October 2015 to 8 October 2015). Heat treatments were applied starting from 09.00 to 16.00 (7 hours) each day. Custom built heat chambers (0.80 m x 1.2 m x 1.0 m height) were placed between two variety sub-plots in each main plot. The target temperature was 40°C inside the chamber. The temperature and relative humidity were logged using a MiniDatalogger (Microlite-PRO-RH, Fourtec Fourier Technologies Ltd.).

Harvest occurred on 9 November 2015 in both heat-stressed and control plots (quadrate of 4 row by 0.30 m). Above ground biomass and seed yield were measured after drying at 40°C for 72 hours. Total N and 15N were determined from these dried tissues using LECO Tru Mac Elemental Analyser (LECO Corporation, St. Joseph, MI, USA) and isotope ratio mass spectrophotometry (IRMS, Hydra 20–20, SerCon), respectively. Amount of N2 fixed from atmosphere (percentage of N derived from atmosphere, %Ndfa) was determined by 15N natural abundance methods according to Unkovich et al. (1994) using wheat as a reference crop grown adjacent to lentil plots. N concentration is expressed as mg N per g seed, while crop N content is the product of the N concentration and the total biomass.

Results

Elevated CO_2 significantly increased total N content in aboveground biomass compared to a[CO_2] (Figure 1A). Heat shock significantly reduced total N content of lentil tested (Figure 1A). PBA Ace had higher (11%) total N content under heat compared with HS3010. Soil N uptake was significantly different between heat stress and control treatments (Figure 1A). The interaction of CO_2 and cultivar was also significant for soil N uptake.

There was a significant effect of e[CO_2] on the amount of fixed N (Figure 1A). Fixed N was higher (10%) under e[CO_2] than a[CO_2]. PBA Ace fixed 23% more N from the atmosphere than HS3010. Nitrogen fixation (fixed N) was reduced significantly ($P<0.05$) by heat stress. Both cultivars also showed significant ($P<0.001$) difference in total fixed N. Percentage of nitrogen derived from atmosphere (%Ndfa) was significantly different between CO_2 treatments, cultivars and heat treatments (data not shown).

Heat stress reduced seed N concentration (Figure 1B) and the interaction between CO_2 and heat was significant. However, e[CO_2] increased the seed N content (17%) for both cultivars (Figure 1C). PBA Ace had 53% higher grain N content compared to HS3010. Heat treatment significantly reduced (36%) the seed N content for both cultivars. There was a significant interaction between cultivar and heat treatment. PBA Ace reduced seed N content by 25 % and HS3010 by 15 % under heat stress. Heat stress significantly reduced the seed yield (Figure 1D).This reduction was more apparent in PBA Ace (50%), regardless of [CO_2]. HS3010 had a 50% reduction in seed yield under a[CO_2] and 30% under e[CO_2]. The yield penalty was negligible when a[CO_2] controls were compared with e[CO_2] heat treatment.

Discussion

In support of our hypothesis, e[CO_2] stimulated N_2 fixation of legumes and increased proportion of symbiotically fixed N contributed to greater total N content for both lentil cultivars under e[CO_2]. The stimulatory effect of e[CO_2] on N_2 fixation can be explained by either greater nodule biomass or greater nodule activity or both (Schortemeyer et al. 2002). Similar findings were reported in *Glycine max* (L.), *Trifolium repense* (L.), *Pisum sativum* (L.) under FACE conditions (Rogers et al. 2009; Butterly et al. 2015; Leakey et al. 2009).

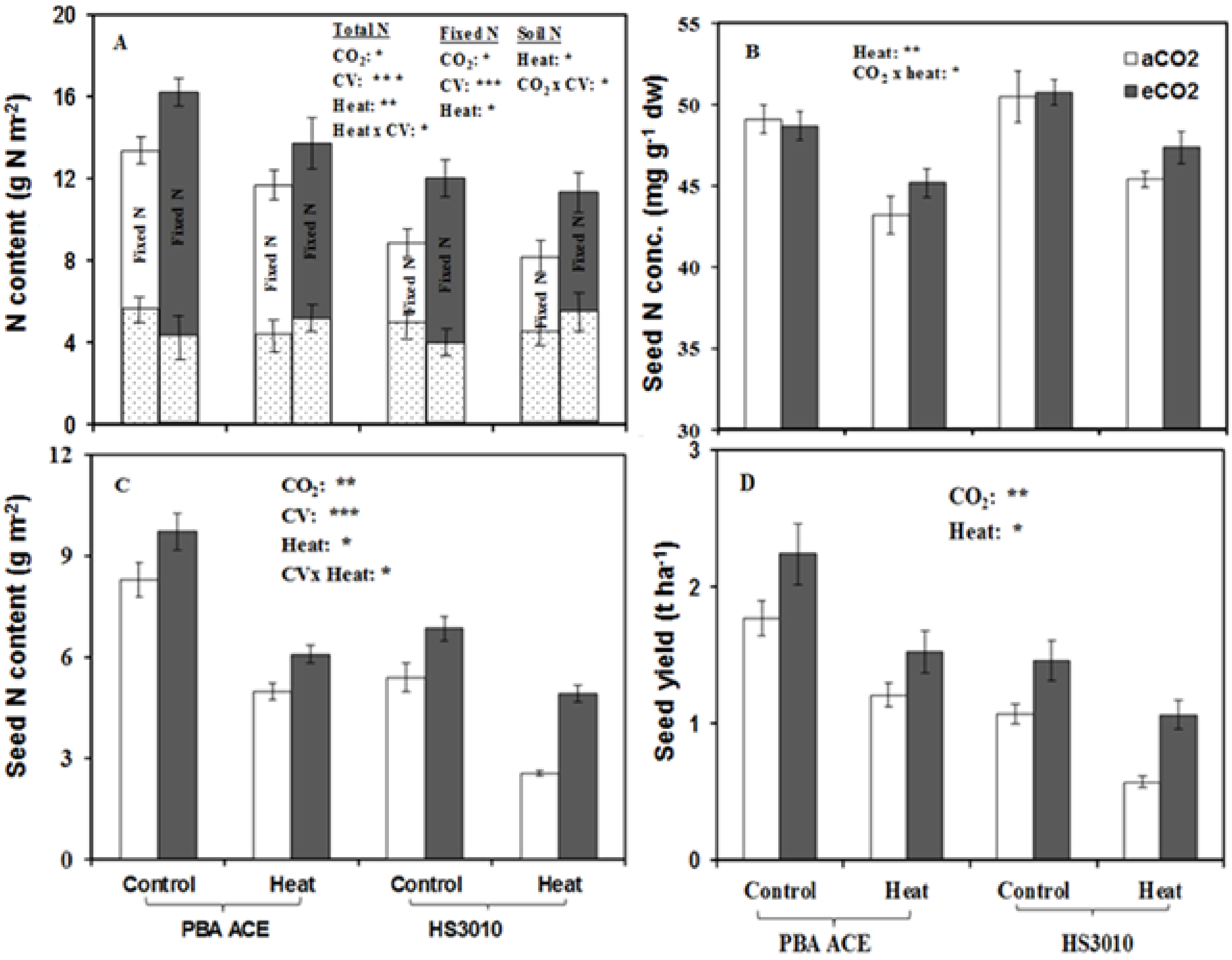

Figure 1. (A) Above ground plant N content (fixed N- plant N from fixation and soil N – plant N taken up from soil), (B) Seed N concentration, (C) Seed N content and (D) Seed yield of two lentil cultivars from final harvest (both control and heat plots) grown under ambient (~ 400 ppm, open bars) or elevated (~ 550 ppm, filled bars) CO_2 concentrations in the Australian Grains Free Air Enrichment (AGFACE) facility, Horsham, Australia. Soil N uptake was shown as dotted bar in Figure A. Data points represent mean values and standard errors of n=4 replicates. P values indicate significance of the effect of CO_2, treatment (Heat), cultivar (CV) as well as their interaction, only significant (* p>0.05, ** P>0.01, *** P>0.001) relations were shown.

Exposure of lentils to heat waves reduced nitrogen fixation compared to the control and a greater reduction was detected under a[CO_2]. Higher temperature generally limits nodule activity, enhances nodule senescence and impairs N fixation (Aranjuelo et al. 2007). Studies supported that heat stress affected nodule formation, function and structure along with nitrogen fixation efficiency of legumes, for example Kurdali (1996) for chickpea. Both lentil cultivars took up more soil N under heat stress as the proportion of N from soil was greater, because less N is fixed. Elevated CO_2 grown lentils may need less soil N compared to a[CO_2], because of higher N_2 fixation. Among two cultivars, PBA Ace fixed more N and depended less on soil N uptake. In addition to fixation, total N content was reduced by heat treatment. The observed reduction of total N content can be explained by a decrease of total biomass (data not shown) under heat stress.

Reduction of seed N concentration by heat stress of both lentil genotypes reduced the seed protein concentration. Regardless of genotypes, there was a higher seed N concentration under e[CO_2] compared to a[CO_2] and if subjected to heat stress this decline may be smaller. This suggest that lentil can maintain seed protein concentration when grown under e[CO_2] (Rogers et al. 2009). Decrease of seed yield of lentils under heat stress has been linked to the poor reproductive developments including fewer pods numbers and lower seed weight (Kaushal et al. 2016). The reduction of seed weight noted here with lower pod numbers (data not shown) under heat stress in both genotypes. As the heat shock was imposed during flat pod development stage, higher temperature causes flower abortion and impairs pollen viability and that lowers total pod numbers in final harvest. As results, these combined effects reduced seed weight and finally seed yield of lentil under heat stress.

Conclusion

Heat stress decreased N yield for both investigated cultivars. However, the negative effects of heat stress are buffered by e[CO_2] in investigated lentils compared to a[CO_2] and allows a partial maintenance of seed N concentration and N_2 fixation.

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