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Research paper

Yield, growth and grain nitrogen response to elevated CO_2 in six lentil (*Lens culinaris*) cultivars grown under Free Air CO_2 Enrichment (FACE) in a semi-arid environment

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ABSTRACT

Atmospheric CO_2 concentrations ([CO_2]) are predicted to increase from current levels of about 400 ppm to reach 550 ppm by 2050. The direct benefits of elevated [CO_2] (e[CO_2]) to plant growth appear to be greater under low rainfall conditions, but there are few field (Free Air CO_2 Enrichment or FACE) experimental set-ups that directly address semi-arid conditions. The objectives of this study were to investigate the following research questions: 1) What are the effects of e[CO_2] on the growth and grain yield of lentil (*Lens culinaris*) grown under semi-arid conditions under FACE? 2) Does e[CO_2] decrease grain nitrogen in lentil? and 3) Is there genotypic variability in the response to e[CO_2] in lentil cultivars? Elevated [CO_2] increased yields by approximately 0.5 t ha^{-1} (relative increase ranging from 18 to 138%) by increasing both biomass accumulation (by 32%) and the harvest index (by up to 60%). However, the relative response of grain yield to e[CO_2] was not consistently greater under dry conditions and might depend on water availability post-flowering. Grain nitrogen concentration was significantly reduced by e[CO_2] under the conditions of this experiment. No differences were found between the cultivars selected in the response to elevated [CO_2] for grain yield or any other parameters observed despite well expressed genotypic variability in many traits of interest. Biomass accumulation from flowering to maturity was considerably increased by elevated [CO_2] (a 50% increase) which suggests that the indeterminate growth habit of lentils provides vegetative sinks in addition to reproductive sinks during the grain-filling period.

1. Introduction

Atmospheric CO_2 concentrations ([CO_2]) have been increasing from about 280 ppm to 406 ppm from the pre-industrial era until now (January 2017; www.co2.earth; last accessed 23 February 2017). If global greenhouse gas emissions remain at the 2010 level, then atmospheric CO_2 concentrations ([CO_2]) should reach 550 ppm by 2050 (IPCC, 2014). This increase in the substrate of photosynthesis has direct implications for plant metabolism, such as increased growth and yield, at least in C3 plants and in the absence of changes in temperature and rainfall patterns (Ainsworth and Long 2005; Ziska et al., 2012). Elevated [CO_2] (e[CO_2]) also reduces stomatal conductance, leading to higher transpiration efficiency (Leakey et al., 2009; Tausz-Posch et al., 2013) and potentially increased crop water productivity under conditions of water stress (Deryng et al., 2016; Gifford, 1979). In environments prone to severe terminal drought (where the crop progressively runs out of water with no resupply), there are however concerns that the early increases in leaf area and biomass accumulation under e[CO_2] might negate the water savings from higher transpiration efficiency, potentially leading to an earlier onset of drought. This could reduce post-flowering growth and translocation of assimilates and therefore reduce the yield response to e[CO_2]. There are few FACE experimental set-ups that directly address this type of water stress, and experimental data to support or disprove this theory is sparse (Deryng et al., 2016).

Higher growth and grain yield are often associated with decreased

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grain quality, especially in cereals, where decreases in grain nitrogen concentration ([N]) and therefore protein concentrations, raise concerns about nutrition and product quality (Jablonski et al., 2002; Myers et al., 2014). Decreased grain [N] under e[CO_2] is preceded by decreases in [N] in vegetative biomass, particularly in leaves (Leakey et al., 2009). There are several hypotheses to explain this decrease in tissue [N] (reviewed by Taub and Wang, 2008). The dilution hypothesis contends that N supply fails to keep up with the increased demand from stimulated biomass growth. Legumes, with their nitrogen-fixing symbionts, would be able to overcome such limitation because the additional carbohydrate acquired under e[CO_2] could feed the N-fixing symbiosis (Rogers et al., 2009). Indeed, field grown soybean did not show decreases in leaf [N] from about mid-season onwards, once the N-fixing symbiosis had established (Rogers et al., 2006). Studies in chambers or high rainfall agro-ecosystems suggested that, in contrast to cereals, legume grains maintain protein concentrations under e[CO_2] (Jablonski et al., 2002; Taub et al., 2008). As N-fixation is more sensitive to water stress than biomass accumulation and leaf expansion (Serraj et al., 1998), in semi-arid environments N-fixation is likely interrupted by water stress and therefore biomass dilution of [N] might occur. Some recent reports have found small but significant decreases in grain protein in legumes which might be partially explained by water stress (Lam et al., 2012 in chickpea (*Cicer arietinum*); Bourgault et al., 2016 in field pea (*Pisum sativum*)).

It might be possible to take advantage of rising atmospheric [CO_2] by selecting for greater CO_2 responsiveness in crop breeding programs either directly or by selecting traits that are associated with a greater response (Ainsworth et al., 2008; Tausz et al., 2013; Ziska et al., 2012). Intraspecific variability in the response to e[CO_2] − a prerequisite for this approach − has been reported in soybean (*Glycine max*): Bishop et al. (2015) have found consistent differences in grain yield response to e[CO_2] among 18 cultivars grown under Free Air CO_2 Enrichment (FACE) and suggested this is a heritable trait. They also suggested that high e[CO_2] response is related to a greater harvest index and a short stature. Similarly, Bunce (2008) investigated variability in the response to e[CO_2] of 4 common bean (*Phaseolus vulgaris*) cultivars and suggested that greater grain yield response was associated with the ability to produce more pods under e[CO_2]. Similarly, Ziska et al. (2001) showed that greater yield response in soybean was related to the ability of some cultivars to increase the seed production on auxiliary branches. In contrast, studies reporting a lack of response to e[CO_2] in one cultivar have often related this to limitations in the ability to use the additional carbohydrates. For example, Sicher et al. (2010) found that a dwarf cultivar of soybean did not show yield increases under e[CO_2]. Grains of this cultivar were 75% smaller and had lower oil seed content than grains of normal cultivar. Taken together, these studies suggest that the response to e[CO_2] might depend on both the capacity of the plant to utilise the additional carbohydrates produced under e[CO_2] and the effective translocation of resources to grains later in yield formation.

Lentil (*Lens culinaris*) is one of the oldest cultivated crops in the world and its production has more than quadrupled since the 1960s reaching a global production of over 5 million tonnes in 2013 (FAOSTAT, 2016). It is well-known as a nutritious grain and forms the basis of many traditional Asian and Middle Eastern recipes (Raghuvanshi and Singh, 2009). In Australia, it is grown as a winter crop under non-irrigated conditions, and therefore frequently subjected to terminal drought conditions. Further, the crop is often exposed to low temperatures during the vegetative stage and high temperature stress by pod filling (Materne and Siddique, 2009). Substantial efforts in breeding lentils in Australia are recent and expanding genetic variability is still seen as a major activity (Siddique et al., 2013). Intraspecific variability in many traits of interest is adequate and could be used in breeding programs (Erskine et al., 2009), although there is little published information on drought tolerance characteristics and no information on potential intraspecific variability in the response to e[CO_2] in lentils.

In this study, we grew a range of lentil lines over three seasons in the Australian Grains FACE (AGFACE) facility. AGFACE is located in the south-eastern Australian grain cropping belt, with a typical semi-arid Mediterranean climate, making it a representative site for significant areas of global lentil production (Materne and Siddique, 2009). Therefore, this experimental set-up allowed us to address the following research questions:

- What are the effects of e[CO_2] on the growth and grain yield of lentil (*Lens culinaris*) grown under realistic drought conditions under Free Air CO_2 Enrichment (FACE)?
- Does e[CO_2] decrease grain [N] in lentil?
- Is there genotypic variability in the response to e[CO_2] in lentil cultivars?

2. Materials and methods

2.1. Experimental site and growing conditions

The Australian Grains Free Air CO_2 Enrichment (AGFACE) facility is located near Horsham, Victoria (36°45′07″S 142°06′52″E, 127 m above sea level). The site is cracking clay soil (Vertosol) with approximately 35% clay content at the surface increasing to 60% at 1.4 m depth. Long term average (based on 1981–2010 period) annual rainfall is 435 mm, with approximately 320 mm falling during the winter growing season (from May to November inclusive). Average maximum and minimum temperatures are 17.6 °C and 5.3 °C respectively during the season, with July being the coldest month (Bureau of Meteorology, 2016). Maximum and minimum temperatures as well as rainfall data during the 2013–2015 growing seasons were recorded by an on-site weather station (MEA Premium Weather Station 103, Measurement Engineering Australia, Magill, SA, Australia; Fig. 1). Soil moisture was also monitored in 2014 and 2015, on a weekly basis in two cultivars to a depth of 1 m with a PR2 Profile Probe (Delta-T Devices, Cambridge, UK) (Fig. 2).

Elevated CO_2 levels (target 550 μmol mol^{-1} air) were maintained during daylight hours by injecting pure CO_2 into the air on the upwind side from horizontal stainless-steel tubes so the gas would be carried across the ring. The tubes were positioned about 150 mm above the canopy and raised following the growth of the crop. Concentrations were maintained within 90% target (495–605 μmol mol^{-1} air) for 93–98% of the time. More details on the site and the CO_2 exposure equipment are given in Mollah et al. (2009).

Plots were sown on 5 June 2013, 12 May 2014 and 26 May 2015. Plots were treated according to local practice with pre-emergence herbicides (simazine, dimethenamid-P, trifluralin, isoxaflutole, and/or glyphosate) prior to sowing and with haloxyfob approximately 4–8 weeks after emergence if required. In 2014, the insecticide dimetoate was also used to control aphids. Superphosphate was placed with the seed at sowing at 9 kg P ha^{-1} and 11 kg S ha^{-1} each year. No nitrogen fertilizer was added, but seeds were inoculated with granular pea and lentil inoculant in 2013 and 2014 (Nodulator, BASF Corporation, Research Triangle Park, NC, USA), and peat-based inoculant in 2015 (NoduleN, New Edge Microbials Pty Ltd, Albury, NSW, Australia).

2.2. Experimental design

In each year (2013–2015), there were 4 ambient and 4 elevated CO_2 octagonal main plots organised in bays each containing one ambient and one e[CO_2] plot. The design within each plot differed from year to year. In 2013, plots were 8 m in diameter and split in half for a plus/minus supplemental irrigation treatment. Six cultivars were organised in sub-plots of 2 rows (0.55 m) by 4 m in each half. However, because the supplemental irrigation treatment was omitted during this season