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Acclimation to elevated CO_2 is improved by low Rubisco and carbohydrate content, and enhanced Rubisco transcripts in the G132 barley mutant

Javier Córdoba[a,b], Pilar Pérez[a], Rosa Morcuende[a], José-Luís Molina-Cano[b,1], Rafael Martinez-Carrasco[a,*]

[a] Institute of Natural Resources and Agrobiology of Salamanca, IRNASA-CSIC, Cordel de Merinas 40, E-37008 Salamanca, Spain
[b] IRTA (Institute for Food and Agricultural Research and Technology), Field Crops, Av. Alcalde Rovira i Roure, 191, E-25198 Lérida, Spain

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ABSTRACT

Variations induced by mutagenesis may identify targets for adapting to rising atmospheric CO_2 concentrations. G132 is a barley mutant with strong decreases in photosynthesis, Rubisco and carbohydrate content, but increased Rubisco transcripts. We tested the hypothesis that G132 will record a greater stimulation of photosynthesis and dry mass gain than the wild-type (WT) in response to CO_2 enrichment. A growth chamber experiment compared the mutant G132 and the WT developed in ambient ($\sim$390 μmol mol^{-1}) and elevated (1200 μmol mol^{-1}) CO_2. Photosynthesis, protein and carbohydrate contents, Rubisco and nitrate reductase activities, leaf morphology, plant dry matter and leaf area were determined. The transcriptome responses of fully expanded leaves of G132 and WT to elevated CO_2 were investigated using microarrays and qRT-PCR. Growth in elevated CO_2 stimulated photosynthesis more in G132 than in WT, in association with a lack of Rubisco down-regulation and lower carbohydrate levels in G132. Electron transport-related proteins increased in G132 and decreased in WT. Elevated CO_2 induced more changes in gene expression in WT than G132. Clustering of the corresponding transcripts showed that the expression of genes involved in carbohydrate synthesis and nitrogen assimilation was enhanced, while that for stress-related genes was repressed in both genotypes, and gene expression for jasmonate metabolism was activated in G132. Elevated CO_2 decreased the transcript levels for a greater number of transcription factors in WT than in G132. CO_2 enrichment increased leaf area in G132 but not in WT, and induced greater dry mass increases in the mutant. The low Rubisco and carbohydrate content and enhanced Rubisco transcripts recorded in G132 are traits that may improve adaptation to rising CO_2.

1. Introduction

Long-term records of atmospheric CO_2 concentrations at Mauna Loa Observatory evidence increases from around 315 μmol mol^{-1} in the late 1950s to above 400 μmol mol^{-1} in the present day, with a 2.2 μmol mol^{-1} annual mean growth rate in the last 10 years (Dr. Tans and Dr. Keeling, www.esrl.noaa.gov/gmd/ccgg/trends/). In plants with C3 photosynthesis, the carboxylation enzyme ribulose-1, 5-bisphosphate carboxylase oxygenase (Rubisco) fixes CO_2 at a faster rate with the elevated CO_2 concentrations predicted for the future. With high CO_2 levels, or with low light intensities, where carboxylation is limited by ribulose-1, 5-bisphosphate (RuBP) regeneration, and not by Rubisco activity, CO_2 inhibition of the Rubisco-catalysed RuBP oxygenation also enhances photosynthesis because it releases

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Abbreviations: 1-SST, sucrose: sucrose 1-fructosyltransferase; 6-FEH, fructan 6-exohydrolase; 6-SFT, sucrose: fructan 6-fructosyltransferase; cyt b6, cytochrome b6; Fq'/Fm', PSII operating quantum efficiency; Fru, fructose; Ftn, fructan; Fv/Fm, PSII maximum quantum efficiency; HT1, high temperature 1 protein kinase; J, potential rate of photosynthetic electron transport; LHCI, light harvesting complex I; LHCII, light harvesting complex II; OPP, oxidative pentose phosphate pathway; PEPCASE, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxylase kinase; PGM, phosphoglucomutase; PORA, protochlorophyllide oxidoreductase A; PSI, Photosystem I; PSII, Photosystem II; rbcL, Rubisco large subunit; rbcS, Rubisco small subunit; R_d, mitochondrial respiration rate in the light; Rubisco, ribulose-1 5-bisphosphate carboxylase oxygenase; RuBP, ribulose-1 5-bisphosphate; SLAC1, slow anion channel-associated 1; SLAH3, SLAC1 homologue 3 slow anion channel; TCA, tricarboxylic acid; V_{cmax}, maximum rate of Rubisco-catalysed carboxylation; WT, wild-type.

[*] Corresponding author.

E-mail addresses: rafael.mc@csic.es, rafael.mcarrasco@gmail.com (R. Martinez-Carrasco).

[1] Retired.

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photosynthetic electron transport products and RuBP (Leakey et al., 2009). Moreover, carbon fixation is stimulated by elevated CO_2 in spite of photosynthetic acclimation, consisting of a decrease in Rubisco activity and content after long-term growth under CO_2 enrichment (Pérez et al., 2005; Sage et al., 1989; Vicente et al., 2015). The maximum rate of electron transport leading to RuBP regeneration also decreases at times (Leakey et al., 2009), although growth in elevated CO_2 increased the photochemical efficiency in our earlier studies (Gutiérrez et al., 2009a). A limited sink capacity can lead to photosynthetic acclimation, and diminish the stimulation of carbon fixation by elevated CO_2 (Aranjuelo et al., 2011). It has been suggested that the mechanism involved may be an accumulation of carbohydrates in leaves that represses Rubisco gene expression (Van Oosten et al., 1994), although the correspondence between carbohydrate accumulation and levels of Rubisco transcripts was weak (Nie et al., 1995). Photosynthetic acclimation is associated with a lower nitrogen content in leaves and the whole plant (del Pozo et al., 2007), for which several explanations have been given, including transpiration-limited nitrogen supply and inhibition of nitrate assimilation (Bloom et al., 2014; Taub and Wang, 2008).

Although Rubisco loss and stomatal closure are the most evident responses to rising CO_2 (Long et al., 2004), transcriptomic changes possibly induced indirectly by elevated CO_2 have been found in several plant species. These changes include the induction of genes for enzymes of the tricarboxylic acid cycle (TCA), oxidative pentose phosphate pathway (OPP), glycolysis (Li et al., 2008), starch, sucrose and fructan synthesis (Fukayama et al., 2011; Li et al., 2008; Vicente et al., 2015), cell wall synthesis, signalling, transport, and defence (Ainsworth et al., 2006; Li et al., 2008). The repression of genes for photosynthesis, nitrogen assimilation and photorespiration (Vicente et al., 2015) has also been reported. The proteomic changes described in plants growing in elevated CO_2 involve increases in the content of the ATP synthase, phosphoglycerate mutase (Aranjuelo et al., 2011), oxygen evolving complex 23 kDa protein, myrosinase precursor, luminal-binding protein, nucleoside diphosphate kinase (Bae and Sicher, 2004), phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase (Aranjuelo et al., 2013), among others. In addition to decreases in Rubisco, losses have been reported in contents of Rubisco activase, adenosine diphosphate glucose pyrophosphatase (Aranjuelo et al., 2011) and the chloroplast glyceraldehyde-3-phosphate dehydrogenase precursor (Bokhari et al., 2007). Together with the transcriptomic and proteomic changes and the increase in carbohydrate levels, elevated CO_2 decreases the content in leaves of amino acids, nitrate (Geiger et al., 1999; Vicente et al., 2015), and organic acids (Aranjuelo et al., 2013).

As plant dry matter is predominantly carbohydrate produced by photosynthesis, an increase in atmospheric CO_2 concentration usually translates into biomass gains in cereals (Gutiérrez et al., 2009a; Kimball, 2016; Vicente et al., 2015) and other crops (Bishop et al., 2015), which vary under certain environmental conditions (Ingvordsen et al., 2015; Kimball, 2016). The increase in crop dry matter and yield with elevated CO_2 is generally low (Leakey et al., 2009). Improving photosynthesis adaptation to atmospheric CO_2 enrichment would allow increasing the efficiency of converting intercepted radiation into biomass, which is possibly the only remaining trait for increasing yields (Long et al., 2006) and meeting the increasing demand for food (Tilman et al., 2011). Traditional breeding has not considered the response to rising ambient CO_2 as a selection criterion (Ainsworth et al., 2008), even though genetic improvement may provide a direct strategy for increasing yields through photosynthesis enhancement (Ziska et al., 2012). There have been several studies on genetic variability in photosynthesis and on yield responses to CO_2, but no correlation has been found between photosynthesis and yield variations (Bishop et al., 2015; Driever et al., 2014). In turn, mutagenesis has been used to generate and increase variability in crop species, and eventually modify plant traits (Batista et al., 2008). There are numerous mutants with altered chlorophyll content and changes in photosynthetic rate and Rubisco content (Li et al., 2013). Some stay-green mutants record a slower degradation of chlorophyll, increased antioxidant capacity, and enhanced photosynthetic competence (Luo et al., 2013; Tian et al., 2013). Moreover, a rice mutation affecting a cytochrome P450 involved in brassinosteroid biosynthesis has erect leaves that increase light capture for photosynthesis and grain yields (Sakamoto et al., 2006). We have reported the characterization of the G132 barely mutant (Córdoba et al., 2016), selected in elevated CO_2 conditions, which shows chlorosis in expanding leaves, low PSII quantum efficiency, and a strong and selective decrease in Rubisco protein and activity, accompanied by increased Rubisco degradation. G132 records increased transcript levels for Rubisco and the PSII light harvesting complex, but lower levels of transcripts for protochlorophyllide oxidoreductase A and psbQ. Despite lower photosynthesis rates and higher intercellular CO_2 concentrations, G132 has greater stomatal conductance than the wild-type (Córdoba et al., 2015). G132 shows Rubisco-limited photosynthesis at very high CO_2 concentrations, and lower non-structural carbohydrate contents than WT. Rubisco-limited photosynthesis has a greater response to CO_2 enrichment than RuBP-limited photosynthesis (Leakey et al., 2009). Moreover, low non-structural carbohydrate levels could minimize photosynthetic acclimation.

The aim of this study was to test the hypothesis that the photosynthesis and growth of the G132 mutant have enhanced responses to elevated CO_2 compared to WT. We selected young plants at the stage of 3–4 leaves unfolded because the larger biomass of older plants in elevated CO_2 often reflects a faster rate of growth at an earlier period of development (Geiger et al., 1998). We have measured the photosynthetic quantum efficiency and gas exchange, and analysed the contents of several photosynthetic proteins, including Rubisco, Rubisco activity and chlorophyll content in both genotypes grown in ambient and elevated CO_2. In addition, we determined the content of soluble carbohydrates and starch, which can trigger the acclimation of photosynthesis to high CO_2. To explore the possible nitrogen limitation of photosynthetic responses to elevated CO_2, we assayed nitrate reductase activity and measured the content of proteins, amino acids and nitrate. The green area and dry matter of plants were recorded to assess the differences in growth responses to CO_2 enrichment. We used genome-wide microarrays and quantitative analyses of selected transcripts to identify genes with a significantly altered expression, as well as possible molecular mechanisms causing the changes between genotypes in the response to elevated CO_2. The ultimate goal was to assist in the identification of targets for adapting to growth under atmospheric CO_2 enrichment.

2. Materials and methods

2.1. Plant material, growing conditions and experimental factors

Barley (*Hordeum vulgare* L.) seeds of the homozygous genotype Graphic as wild-type (WT) and homozygous sodium azide-mutagenized seeds of the G132 line, grown and selected in 2000 $\mu mol\,mol^{-1}$ CO_2, were obtained from Dr J. L. Molina-Cano's mutant collection at the IRTA research institute. The G132 seed stock was backcrossed once to Graphic (BC1F2), and this material was subjected to the reported experiments. The study of the exome capture of G132 compared with WT is currently a work in progress. The seeds were surface sterilized with hypochlorite, and sown in 5 L pots with 1.2 kg of peat: perlite (3:1) substrate, with a density of four plants per pot after emergence (completed in both genotypes