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Functional and transcriptional characterization of a barley mutant with impaired photosynthesis

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ABSTRACT

Chemical mutagenesis induces variations that may assist in the identification of targets for adaptation to growth under atmospheric CO_2 enrichment. The aim of this work was to characterize the limitations causing reduced photosynthetic capacity in G132 mutagenized barley (*Hordeum vulgare* L. cv. Graphic) grown in a glasshouse. Compared to the wild type (WT) G132 showed increased transcript levels for the PSII light harvesting complex, but lower levels of chlorophyll, transcripts for protochlorophyllide oxidoreductase A and psbQ, and PSII quantum efficiency in young leaves. Rubisco limitation had an overriding influence on G132 photosynthesis, and was due to strong and selective decreases in Rubisco protein and activity. These reductions were accompanied by enhanced Rubisco transcripts, but increased levels of a Rubisco degradation product. G132 showed lower levels of carbohydrates, amino acids and corresponding transcripts, and proteins, but not of nitrate. Many of the measured parameters recovered in the mutant as development progressed, or decreased less than in the WT, indicating that senescence was delayed. G132 had a longer growth period than the WT and similar final plant dry matter. The reduced resource investment in Rubisco of G132 may prove useful for studies on barley adaptation to elevated CO_2 and climate change.

1. Introduction

The development of new crop varieties and agronomic practices led to an increase in food production in the 1960s and 1970s, but several recent studies indicate that yields may no longer be increasing in different regions of the world [1]. The demand for crops has similarly being increasing since 1960, and may rise by 100–110% between 2005 and 2050 [2]. Yields in four major crops – maize, rice, wheat, and soybean – are increasing at 0.9–1.6% per year, which is less than the annual rate of 2.4% required to double global production by 2050 [1]. Climate trends can account for ∼10% of the yield stagnation observed in Europe over the last two decades; thus, temperature and precipitation changes in Europe have reduced average wheat and barley yields by 2.5% and 3.8%, respectively [3]. Consistent with these climate effects on crops a study on 138 spring barley accessions found that grain yield decreased by 29%, with a concurrent rise in temperature (+5 °C) and CO_2 concentration (700 μmol mol^{-1}), in spite of the 16% yield increase caused by elevated CO_2 [4]. In addition to changes in agricultural subsidies and environmental policies [3], proximity to the theoretical peak in biomass allocation to the harvestable part of agricultural crops is a likely explanation for declining yield increases [5,6]. Extending the cultivated land area is not a sustainable option, and thus the productivity of existing arable land will have to be improved. It is generally accepted that the only way to improve yield

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Abbreviations: A, rate of CO_2 assimilation; A_c, rate of CO_2 assimilation limited by Rubisco; A_j, , rate of CO_2 assimilation limited by RuBP regeneration; Chl, chlorophyll; C_i, intercellular CO_2 concentration in leaves; FEH, fructan exohydrolase; Fv/Fm, maximum quantum efficiency of PSII photochemistry; Fq'/Fm', PSII operating efficiency; Φ_{NPQ}, quantum yield of non-photochemical quenching; g_s, stomatal conductance; J, potential rate of photosynthetic electron transport; LHCII, PSII light-harvesting complex; PORA, protochlorophyllide oxidoreductase A; PSI, photosystem I; PSII, photosystem II; qL, fraction of PSII centres which are in the open state; qRT-PCR, quantitative real-time polymerase chain reaction; rbcL, Rubisco large subunit; R_d, mitochondrial respiration rate in the light; RuBP, Ribulose-1, 5-bisphosphate; Rubisco, Ribulose-1,5-bisphosphate carboxylase/oxygenase; SLA, specific leaf area; 1-SST, sucrose: sucrose 1-fructosyltransferase; 6-SFT, sucrose: fructan 6-fructosyltransferase; SBPase, sedoheptulose-1,7-bisphosphatase; TPU, triose-phosphate use; V_{cmax}, maximum rate of Rubisco-catalyzed carboxylation; WT, wild-type.

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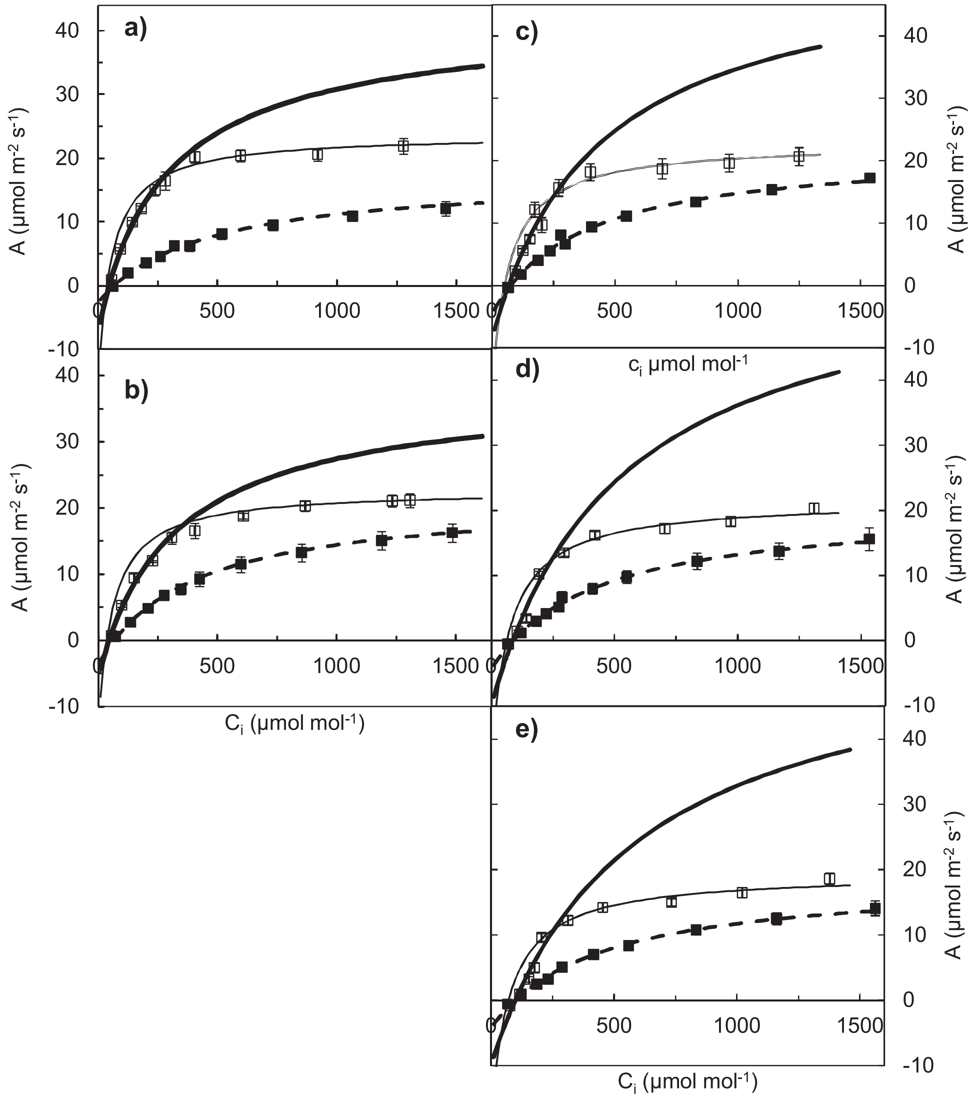

Fig. 1. Rate of CO$_2$ assimilation limited by Rubisco (A_c, thick lines) and by RuBP regeneration (A_j, thin line) in barley (*Hordeum vulgare* L.). Measured and fitted values for the G132 mutant (closed symbols, broken line) and Graphic WT (open symbols, solid lines). There was no RuBP-limited CO$_2$ assimilation in G132 (see text for details). Curves were fitted with the LeafWeb (leafweb.ornl.gov) utility to five replicate leaves per genotype and the mean values are presented. Measurements in (a, c) the youngest fully expanded leaf (leaf *a*), and (b, d) the penultimate (leaf *b*) and (e) antepenultimate (leaf *c*) leaves of plants with (a, b) 3-4 leaves unfolded (13-14 growth stage, Zadoks scale) and (c–e) 5–7 leaves unfolded (15–17 stage), with the WT being more developed. Vertical bars represent twice the standard error of means. The corresponding V_{cmax} and J values and probability in the analysis of variance are shown in Table 2.

potential is through an enhancement of radiation use efficiency [6]. Studies with crops grown under elevated CO$_2$ conditions [5] and model simulations [7] indicate that increasing photosynthetic performance may raise efficiency in energy conversion and increase crop yields to meet global food demand [8] in the future's, CO$_2$-rich atmosphere.

As CO$_2$ increases, photosynthesis in C$_3$ crops shifts from a Ribulose-1,5-bisphoshate carboxylase oxygenase (Rubisco) limitation to a Ribulose-1,5-bisphosphate (RuBP)-regeneration limitation [9]. Therefore, one way to improve crop adaptation to atmospheric CO$_2$ enrichment may be to increase the capacity for RuBP-regeneration to match the enhanced rates of carboxylation. Similarly, there is evidence of increases in the RuBP-regeneration capacity of crops under elevated CO$_2$. Thus, fructose-1, 6-bisphosphatase levels increased in *Lolium perenne* grown at high nitrogen supply [10], and the content of Rubisco decreased in tobacco while that of other Calvin–Benson enzymes increased [11]. The impacts on photosynthesis of changes in the activity of several Calvin–Benson cycle enzymes have been assessed in studies of antisense transformants. Transgenic plants with more than 50% reductions in the amounts of carbon-reduction-cycle enzymes have shown that some of them are present in excess. Included among these enzymes are glyceraldehyde 3-phosphate dehydrogenase [12], fructose-1, 6-bisphosphatase [13], phosphoribulokinase [14], plastid aldolase [15] and, under moderate light, Rubisco, but not under high light intensity [16]. In contrast, photosynthesis is sensitive to small reductions in transketolase [17] and sedoheptulose-1,7-bisphosphatase (SBPase [18]). Photosynthesis is inhibited by decreases in contents of cytochrome b_6f, which is involved in photosynthetic electron transport, and thus in RuBP-