Optimizing RuBP regeneration to increase photosynthetic capacity

Martin Parry[1], Pippa Madgwick[1], Elizabete Carmo-Silva[1], Christine Raines[2],

[1]Rothamsted Research, UK; [2]University of Essex, UK; [3]CIMMYT, Mexico

Abstract

The regeneration of RuBP is a major factor limiting photosynthesis at sub-saturating light levels. Optimizing this process by overexpressing the enzymes sedoheptulose-1,7-bisphosphatase (SBPase) and fructose-1,6-bisphosphate aldolase (FBP aldolase) is predicted to increase wheat photosynthetic capacity. With combined funding from the BBSRC and CIMMYT, transgenic lines have been produced to overexpress either SBPase or FBP aldolase in a common UK wheat cultivar and in two CIMMYT lines. Current efforts are characterizing the most promising lines – that is, with considerably more SBPase or FBP aldolase – for further studies of the effects on photosynthetic capacity.

Introduction

Photosynthesis is co-limited in the canopy by the kinetics of Rubisco and the regeneration rate of its substrate, ribulose-1,5- bisphosphate (RuBP). RuBP regeneration is particularly limiting when leaves operate at sub-saturating light, which occurs frequently in a canopy environment. The two limiting enzymes in RuBP regeneration are sedoheptulose-1,7-bisphosphatase (SBPase) and fructose-1,6-bisphosphate aldolase (FBP aldolase) (Raines 2003 2006). Increasing RuBP regeneration in model plant species by overexpressing these enzymes substantially increases photosynthesis (Lefebvre et al. 2005). The goal of this project is to produce transgenic wheat to increase photosynthetic capacity though increased supply of RuBP.

Materials and methods

Constructs were made to overexpress SBPase and FBP aldolase in wheat, using a vector designed at Rothamsted Research (Alison Huttly). Biolistic transformation of wheat (*Triticum aestivum*) was carried out by the Cereal Transformation Group at Rothamsted Research (Sparks and Jones 2014). Transgenic plants were produced overexpressing SBPase or FBP aldolase in the common UK wheat cultivar Cadenza as well as in the CIMMYT lines HIST10 and HIST13.

The presence of the SBPase or FBP aldolase transgenes was verified by PCR analyses. Gene expression levels and protein amounts in plants harboring the foreign DNA are being determined by RT-qPCR and Western Blotting. Photosynthetic performance is being measured by the response of net CO_2 assimilation (A) to the intercellular CO_2 concentration (Ci) using an infrared gas analyzer from LICOR (LI-6400XT). Further molecular, biochemical, and physiological studies will include characterization of the amounts of SBPase and FBP aldolase (essentially as in Lefebvre et al. 2005) in the different transgenic lines and the corresponding effects on photosynthetic performance.

Results

Constructs were made to overexpress either SBPase or FBP aldolase in wheat. A number of wheat transformant lines have been produced for the two constructs with the UK common wheat cultivar Cadenza or the CIMMYT lines HIST10 and HIST13 as background (Table 1).

Table 1. Transformant lines of wheat produced using the UK cultivar Cadenza or the CIMMYT lines HIST10 and HIST 13 as background for overexpression of sedoheptulose-1,7-bisphosphatase (SBPase) and fructose bisphosphate aldolase (FBP aldolase). The production of these lines was funded by CIMMYT and the BBSRC, through the CIRC and the 20:20 Wheat® ISP of Rothamsted Research.

Gene of interest	Background cultivar	Transformant lines
SBPase	Cadenza	25
SBPase	HIST10	34
SBPase	HIST13	34
FBP aldolase	Cadenza	37
FBP aldolase	HIST10	36
FBP aldolase	HIST13	18

The first set of T1 FBP aldolase and SBPase transgenic plants have been analyzed for improved photosynthetic performance (Figs. 1 and 2). From the few lines analyzed thus far, only FBP4 showed a potential increase in photosynthetic capacity at elevated CO_2.

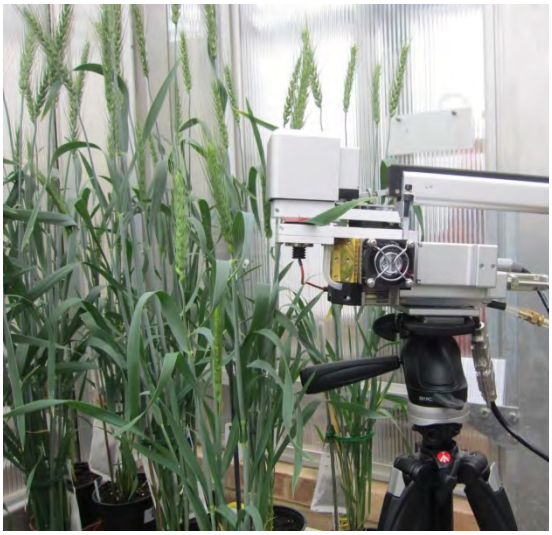

Figure 1. Gas-exchange analyses (LI-6400XT) of wheat T1 transformant plants growing in the glasshouse.

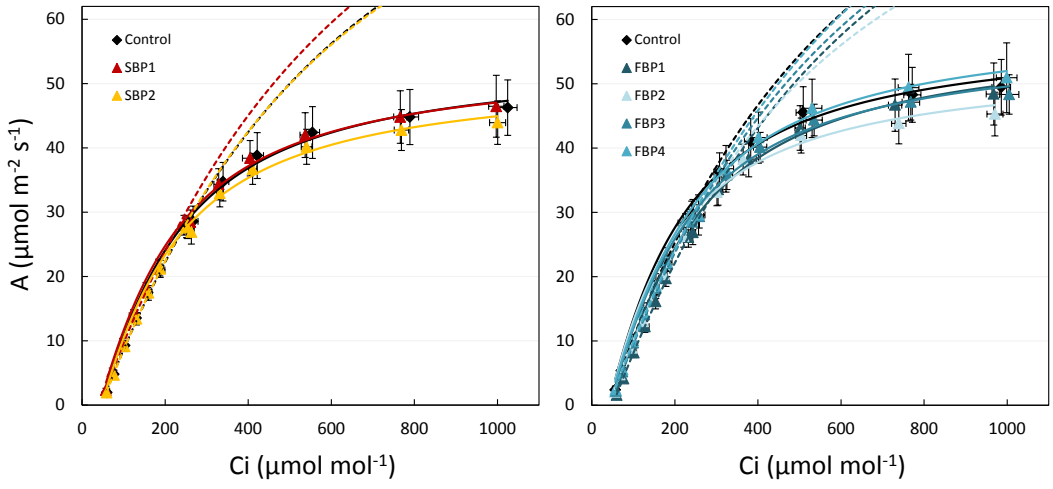

Figure 2. Net CO_2 assimilation (A) response to the intercellular CO_2 concentration (Ci) in T1 transformant plants of 2 lines expressing SBPase and 4 lines expressing FBP aldolase in the CIMMYT background line HIST13 in relation to the respective control.

Preliminary immunoblotting analyses suggest that the line FBP4 above has increased expression of FBP aldolase (10-15%), but there is no clear evidence for increased expression of either SBPase or FBP aldolase in the remaining lines shown in Figure 2. Further analyses will verify these observations and characterize the other lines in Table 1.

Discussion and conclusions

RuBP regeneration limits photosynthetic capacity, particularly at sub-saturating light. Overexpression of the enzymes SBPase and FBP aldolase successfully resulted in increased photosynthesis in model plants (Lefebvre et al. 2005). A large number of wheat transgenic lines were produced to overexpress either of the two enzymes in three different background wheat genotypes, the common UK cultivar Cadenza and the two CIMMYT lines HIST10 and HIST13. The hypothesis is that lines showing considerable overexpression of SBPase or FBP aldolase will have marginally increased photosynthetic capacity, which through the growing season will translate into significant increases in biomass production and wheat yield. The lines with largest expression of either of the enzymes will be crossed to make full use of the potential increase in photosynthetic capacity.

Transformant T_1 and T_2 plants are being characterized for the presence of the transgene, gene expression and protein amounts of SBPase or FBP aldolase and photosynthetic performance. Preliminary data suggesting that increased expression of FBP aldolase results in at least slightly increased photosynthetic capacity warrants further characterization of all the lines in Table 1. Moreover, the comparison of increased RuBP regeneration capacity in the three different wheat background genotypes will indicate whether there is advantage in using either of them for optimizing RuBP regeneration to increase photosynthesis.

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Improving the thermal stability of Rubisco activase

Elizabete Carmo-Silva[1], Joanna Scales[1], Pippa Madgwick[1], Mike Salvucci[2], Christine Raines[3], Martin Parry[1]

[1]Rothamsted Research, UK; [2]USDA, USA; [3]University of Essex, UK; [4]CIMMYT, Mexico

Abstract

The thermal sensitivity of the key photosynthetic enzyme Rubisco activase limits wheat photosynthesis at moderately high temperatures. Introduction of a more thermal tolerant form of Rubisco activase, such as that from cotton, into wheat is predicted to broaden the temperature range of optimal Rubisco activation and photosynthetic CO_2 assimilation. Transgenic lines have been produced to express the cotton Rubisco activase in wheat and current efforts are characterizing the most promising lines for further studies of photosynthetic performance at moderately high temperatures. Joanna Scales, a BBSRC PhD student jointly supervised by Martin Parry, Christine Raines, and Mike Salvucci, generated the transformation constructs and will undertake the molecular and biochemical analysis of the transformant lines. It is predicted that the cotton Rubisco activase will confer superior thermal tolerance to wheat photosynthesis.

Introduction

The primary determinant of crop biomass is cumulative photosynthesis over the growing season. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme; it catalyzes the assimilation of CO_2 through carboxylation of RuBP in the Calvin-Benson cycle. Rubisco is prone to inactivation by non-productive binding of sugar-phosphates to active sites (Parry et al. 2013). Conformational remodeling by the specific chaperone, Rubisco activase, is required to reactivate Rubisco. Rubisco activase is extremely sensitive to elevated temperatures and an important determinant of the heat stability of photosynthesis (Salvucci and Crafts-Brandner 2004; Barta et al. 2010). Importantly, the Rubisco activase from cool-season species, such as wheat, becomes inactive at considerably lower temperatures than the enzyme from warm-adapted species, such as cotton (Carmo-Silva and Salvucci 2011). The goal of this project is to produce wheat germplasm with improved photosynthetic tolerance to heat stress by introducing cotton Rubisco activase into wheat.

Materials and methods

Constructs were made to express cotton Rubisco activase α- and β-isoforms (Salvucci et al. 2003) in wheat, using a vector designed at Rothamsted Research (Dr. Alison Huttly). The two isoforms are being expressed independently because of their different regulatory properties (Carmo-Silva and Salvucci 2013). Biolistic transformation of wheat (*Triticum aestivum* cv. Cadenza) was carried out by the Cereal Transformation Group at Rothamsted Research to generate transgenic plants expressing the cotton Rubisco activase (Sparks and Jones 2014).

The activation of Rubisco purified from wheat by recombinant cotton Rubisco activase was measured in vitro as previously described (Barta et al. 2010). The presence of the cotton Rubisco activase transgene is being verified by PCR analyses. Rubisco activase gene expression levels and protein amounts in plants harboring the foreign DNA are being determined by RT-qPCR and Western Blotting. Further molecular, biochemical, and physiological studies will include characterization of the amounts of cotton and wheat Rubisco activase isoforms in the transgenic lines and the corresponding effects on Rubisco activation and photosynthesis at different temperatures (Carmo-Silva and Salvucci 2012; Scales et al. 2014).

Results

In vitro experiments confirmed that recombinant cotton Rubisco activase competently activates Rubisco purified from wheat. Hence, the duo cotton Rubisco activase and wheat Rubisco is expected to function in vivo. Constructs