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Optimizing RuBP regeneration to increase photosynthetic capacity

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Summary

SP1.5 aims to increase the photosynthetic capacity of wheat by increasing RuBP generation. We received funding to generate initial transgenic lines and analysis of the transformants is underway. Further transgenic events, using alternate promoters, may be needed to optimise expression levels. CIMMYT will conduct crosses of the best lines.

Results and Discussion

Photosynthesis is co-limited in the canopy by the kinetics of Rubisco and the regeneration rate of RuBP (reflecting light harvesting, electron transport, and photophosphorylation). Increasing RuBP regeneration in model plant species substantially increases photosynthesis. The two limiting enzymes in RuBP regeneration are sedoheptulose-1,7-bisphosphatase (SBPase) and fructose bisphosphate aldolase (FBP aldolase) (Raines 2003, 2006), and these are the targets for over-expression in transgenic wheat. The project is high impact with a medium- to long-term delivery timeframe, and therefore has seed funding directly from CIMMYT to allow commencement of transformation experiments.

At Rothamsted Research, we have made constructs to over-express wheat SBPase (pSBPaseT2) and FBP aldolase (pFBPaldT2) under the control of the rice tungro bacilliform virus promoter (RTVP), which targets expression to the leaf lamina. Scutellum of the spring wheat CV Cadenza, and the CIMMYT lines HIST10 and HIST13, have been transformed via biolistics and independent transgenic lines recovered (Table 1).

Table 1. Transgenic lines produced with CIMMYT, CIRC, and 20:20 Wheat® funding

Wheat line	Gene inserted	Number of independent lines
Cadenza	pFBPaldT2	37
Cadenza	pSBPaseT2	25
HIST10	pFBPaldT2	5
HIST10	pSBPaseT2	5
HIST13	pFBPaldT2	12
HIST13	pSBPaseT2	8

The Cadenza transgenic lines generated are now being screened at the University of Essex to determine the amounts of SBPase and FBPaldolase in each line. The Hist10 and Hist13 lines are being screened at Rothamsted Research for improved photosynthetic properties (Fig. 1).

Figure 1. Measuring gas exchange and fluorescence in Hist10-SBPase transgenic lines.

Two new potential photosynthetic tissue-specific (Rubisco activase) promoter fragments from *Brachypodium* were cloned and linked to the β-glucunoridase (GUS) reporter gene. Neither of the two tested promoters successfully drove GUS expression in wheat leaves. Alternative promoters are now being evaluated.

Next Steps

We will confirm gene expression and the amount of proteins in transgenic lines. If increased levels of SBPase and/or FBPaldolase are not observed in the mesophyll cells, we will redesign transformation constructs to achieve this. Once higher levels of SBPase and/or FBPaldolase are achieved in the mesophyll cells, we will determine the impact of increased RuBP regeneration on photosynthetic performance, in each of the genetic backgrounds. Lines with improved performance will then be tested under controlled environments and field conditions.

References

Raines, C.A. 2003. Photosynthesis Research 75:1–10.
Raines, C.A. 2006. Plant, Cell and Environment 29:331–339.