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EVALUATION OF WHEAT MUTANTS FOR THERMO-STABILITY THROUGH PHYSIOLOGICAL PARAMETERS

G.S.S. KHATTAK[1*], M.A.J. PARRY[2], P.J. ANDRALOJC[2], P. SRINIVES[3] and I. SAEED[1]

[1]Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, KPK, Pakistan
[2]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK
[3]Kasetsart University, KamphaengSaen, NakhonPathom, 73140, Thailand
[*]Corresponding author's email: gssktt@yahoo.com

SUMMARY

Photosynthetic activity performance of 3 wheat mutants such as BG48-3-1, BG48-3-7, and BD29-3-9 were studied at 2 different levels of temperature (20 °C and 30 °C) at seedling stage under controlled environment conditions during 2011 at Rothamsted Research, UK. These mutants had earlier been developed from 'Cham1' by exposing germinating seeds to the chemical mutagen, ethyl methane sulphonate (EMS). Simple mutations affecting amino acid residues close to catalytically important regions of Rubisco Activase in these mutants were identified through TILLING (Targeting Induced Local Lesions in Genomes). Although generally the mutants were less active for photosynthetic activity (*A*) than 'Cham1' (control) at 20 °C but their *A* exceeded those of the control at every CO_2 concentration and photosynthetic photon flux density (PPFD) tested at 30 °C. The higher *A* of the mutant lines at 30 °C was correlated with the induction of mutations affecting amino acid residues close to catalytically important regions of Rubisco activase. Differing trends in leaf stomatal conductance (g_s) were observed for mutants and parent lines at 20 °C and 30 °C. Among the mutants, those with g_s below 0.54 mmol m^{-2} s^{-1} at 20 °C showed greater g_s at 30 °C; while the mutants exceeding the g_s above 0.54 mmol m^{-2} s^{-1} at 20 °C showed slightly lower g_s at 30 °C. In Cham1, however, the g_s was invariably higher at 30 °C than 20 °C. Intercellular CO_2 (*Ci*) was slightly lower in all mutant lines at every CO_2 concentration and at all light intensities. *Ci* was also slightly lower in mutants at lowest light intensities at 20 than 30 °C. In contrast, Cham1 showed modest increase in *Ci* at 30 °C. Mutant BG48-3-1 at 30 °C displayed the highest rates of transpiration (*E*), while Cham1 and mutant BG48-3-7 at 20 °C displayed the highest *E*. The results are consistent with Rubisco activase from the mutant lines possessing greater thermal-stability than parent 'Cham1' at 30 °C under different levels of CO_2 and PPFD. However, the mutant lines require more thorough evaluation before recommendation as a source of germplasm with enhanced thermal tolerance for use in wheat breeding programs.

Keywords: Mutants, photosynthesis, Rubisco, thermo-stability, transpiration, wheat

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INTRODUCTION

Increase in biomass partitioning to grain in wheat by the introgression of dwarfing (Rht) genes was achieved with little or no knowledge of the factors governing the genetic variability that breeders exploited for crop improvement. (Borlaug and Dowswell, 2005; Borlaug, 2007). However, further increases in biomass partitioning to grain (increased harvest index, HI) is expected to result in diminishing returns, because the partitioning levels of modern wheat

varieties grown in temperate climates already exceeds 0.50, a value close to the theoretical limit for this trait (Calderini*et al*., 1999). In this situation, further substantial increases in wheat yield will necessitate an increase in total biomass and, therefore, an increase in photosynthesis. Zhu *et al*., (2008) have shown that the theoretical photosynthetic energy conversion efficiency for C3 plants under ideal field conditions is approximately 4.6%. However, the actual energy conversion efficiency is usually less than one-third of this theoretical value. Thus there is significant potential for increasing photosynthetic energy conversion efficiency (Reynolds *et al*., 2000; Zhu *et al*., 2008). There are a range of approaches by which this could be realized (Long *et al*., 2006; Zhu *et al*., 2010). Increasing the photosynthetic rate per unit leaf area is one strategy identified for wheat (Long *et al*., 2006; Raines, 2006; Parry *et al*., 2007). However, photosynthesis is particularly sensitive to inhibition by moderate heat stress and this inhibition correlates with a decrease in the activation state of Rubisco and generally translates into a decrease in yield (Salvucci and Crafts-Brandner, 2004). An activase limitation occurs when the capacity of Rubisco to consume RuBP is reduced by deactivation of catalytic sites to such an extent that RuBP consumption capacity falls below RuBP regeneration capacity (Sage *et al*., 2008). *In vitro,* the ability of activase to maintain or promote Rubisco activation decrease with temperature but is not affected by CO_2 (Jensen, 2000). Rubisco activation requires a specific chaperone, Rubisco activase, which has a relatively low temperature optimum for reactivating Rubisco (Parry *et al*., 2011) and whose activity declines precipitously under moderate heat stress. It has earlier been demonstrated in Arabidopsis that thermo-tolerance of photosynthesis can be improved by increasing the thermal stability of Rubisco activase (Kumar *et al*., 2009). Improving the thermal stability of Rubisco activase in wheat in a similar way, could improve the performance of this crop at elevated temperature.

The thermo-stability of three wheat mutants with simple mutations affecting amino acid residues close to catalytically important regions of Rubisco Activase, identified by means of TILLING, were studied at 20 °C and 30 °C in a controlled environment facility and the results are reported in the current manuscript.

MATERIALS AND METHODS

Three Wheat mutants namely BG48-3-1, BG48-3-7 and BD29-3-9 with simple mutations affecting amino acid residues close to catalytically important regions of Rubisco Activase induced through exposure of germinating seeds of wheat genotype 'Cham1' to the chemical mutagen, ethyl methane sulphonate (EMS) were identified via TILLING at Rothamsted Research, UK. The mutants along with parent were planted, 5 seeds per pot, in a completely randomized experimental design with 4 replications under controlled environment conditions in a growth cabinet at 20 and 30 °C during 2011. Soil composition was a composite of Rothamsted Nematode Mix, Rothamsted Weed Mix and Rothamsted Prescription mix (compost). The growth conditions were chosen to increase the likelihood of identifying phenotypic differences between the chosen genotypes. Specifically, alternating periods of light and darkness, each of 1 h duration, throughout the day except between 10 am and 2 pm: when continuous illumination took place to enable sufficient time for establishment of steady state photosynthesis and then to make the required measurements. Light intensity and humidity were 700 µmol m^{-2} s^{-1} and 70%, respectively.

Photosynthetic activity (net CO_2 assimilation rate or photosynthesis, *A*), leaf stomatal conductance to H_2O (g_s), and leaf intercellular CO_2 (*Ci*) and transpiration rate (*E*) were measured using a portable infrared gas analyser (Li-Cor 6400 XT) at seedling stage, 3 weeks after planting. Measurements were recorded on a single leaf per plant for each genotype. Cuvette conditions were: reference CO_2, 100, 200, 400, 500, 650, 800 and 1000 (µmol CO_2 mol air^{-1}) at a Photosynthetic Photon Flux Density (PPFD) of 1500 (µmol photons m^{-2} s^{-1}); PPFD, 0, 100, 250, 500, 1000 and 1500 (µmol photons m^{-2} s^{-1}) at reference CO_2 of 400 (µmol CO_2 mol air^{-1}); leaf temperature, 22 ± 0.5 (mean ± SD) $^{\circ}$C; relative humidity, 60% and the

cuvette fan was set to fast. The *PPFD* measurements for mutant BG48-3-1 were measured only in a growth cabinet having 30 °C and missed data recording in a growth cabinet having 20 °C therefore; this mutant data has not been included in the PPFD measurements.

RESULTS

Effect of temperatures (20 °C and 30 °C) on IRGA's measurements at different CO_2 concentrations

Photosynthetic activity, stomatal conductance to H_2O, transpiration and leaf intercellular CO_2 of the studied wheat mutants and their parent at different CO_2 concentration grown under controlled environments at 20 and 30 °C (Tables 1 to 4). Mutants showed higher *A* at 30 °C compared to 20 °C, while the parent Cham1 showed higher *A* at 20 °C compared to 30 °C. In case of g_s, the mutants BG48-3-1 and BG48-3-7, and parent Cham1 showed higher g_s at 30 °C compared to 20 °C while mutant BD29-3-9 showed higher g_s at 20 °C than 30 °C. Higher *A* was observed at 30 °C by mutants and parent. Mutants and Cham 1 showed higher *Ci* at 20 and 30 °C, respectively.

Effect of temperature (20 and 30 °C) on IRGA's measurements at different photosynthetic photon flux densities (PPFD)

The studied physiological parameters were also determined at different *PPFD* (µmol photon $m^{-2}s^{-1}$) as shown in Tables 5 to 8. The mutant BG48-3-7 and parent (Cham1) showed higher *A* at 30 °C and 20 °C, respectively, while mutant BD29-3-9 behaved differently at different *PPFD* at 20 °C and 30 °C. Mutant BG48-3-7 and BD29-3-9 showed higher g_s at 30 °C and 20 °C, respectively. Mutants BG48-3-7 and BD29-3-9 and parent had higher *E* at all PPFD levels at 30 °C compared to 20 °C. Mutants BG48-3-7 and BD29-3-9 had higher '*Ci*' at all PPFD levels at 20 °C while Cham 1 showed higher '*Ci*' at 0 and 100 PPFD at 20 °C but higher at 250, 500, 1000 and 1500 PPFD levels under 30 °C.

DISCUSSION

The low *A* of parent 'Cham1' at 30 °C compared to 20°C revealed its Rubisco activase thermo-sensitivity at high temperature. Rubisco activase is a chaperone which promotes and maintains the catalytic activity of Rubisco. Physiologically, Rubisco activase plays a vital role in the response of photosynthesis to temperature (Portis, 2003). The high *A* of the evaluated wheat mutants at 30 °C compared to 20 °C are consistent with a Rubisco activase with greater thermo-stability at elevated temperature than the parent line. Rubisco deactivation (thermo-sensitivity of Rubisco activase) at thermal optimum has also been observed in Arabidopsis (Salvucci*et al*., 2006), spinach (Yamori*et al*., 2006), wheat (Law and Crafts- Brandner, 1999), cotton (Crafts-Brandner and Law, 2000), tobacco (Sharkey *et al*., 2001), pea (Haldimann and Feller, 2005), and rice (Makino and Sage, 2007).

In agreement with the trends revealed in Tables 3 and 7, Law and Crafts-Brandner (1999) demonstrated that *E* increased with increasing temperature in wheat at an approximate rate of 5 mmol m^{-2} s^{-1} per 10 °C rise in temperature, over the range 20 – 30 °C. However, the wheat *Ci* has been reported to increase modestly (280-300 µmol CO_2. mol air^{-1}) over a similar temperature (Kobza and Edwards, 1987) (Tables 4 and 8).

The higher g_s and *E* of all mutants and their parent 'Cham1' at 30 °C than 20 °C clearly indicates that stomatal openings at high temperature were allowing an efficient cooling of leaves even *A* of 'Cham1' was low at 30 °C compared to 20 °C because of the Rubisco activase thermo-sensitivity at high temperature. Under the sufficient water availability in the soil, Feller (2006) has also reported high stomatal opening and *E* in bean crop for allowing an efficient cooling of the leaf even in the shadow where *A* was low. Similarly, it has already been reported that reduction in *A* in higher plants is not necessarily linked with increase or decrease in g_s and *E* (Heng*et al*., 2004; Parry *et al*., 2011).

The mutant BD29-3-9 at higher CO_2 concentration showed increased *A* at 30 °C followed by mutants BG48-3-7 and BG48-3-1. The variation observed in the *A* at higher temperature among mutants was surprising since

all the mutants had the same point mutations affecting amino acid residues close to catalytically important regions of Rubisco activase. It is possible that the chemical mutagenesis also gave rise to the differences in the capacity of electron transport to regenerate RuBP among the mutant lines. The evaluated mutants could be recommended as the useful wheat germplasm for improving thermal stability of Rubisco activase, but will require more thorough evaluation before recommendation as a source of germplasm with enhanced thermal tolerance for use in wheat breeding programs.

Table 1. Photosynthetic activity (µmol $m^{-2}s^{-1}$) of 3 wheat mutants and their parent at PPFD of 1500 (µmol photons m^{-2} s^{-1}) as a function of CO_2 (µmol CO_2 mol^{-1} of the air) concentration at 20 and 30 °C.

CO_2 Concentration	BG48-3,1		BG48-3,7		BD29-3,9		Cham 1 (Parent)	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
100	1.52	2.35	3.00	0.92	4.60	2.12	5.18	1.21
200	7.57	11.21	6.85	9.02	12.24	11.48	16.92	7.40
400	15.66	26.19	12.16	24.74	25.37	30.13	36.26	20.01
500	16.68	31.70	16.22	30.44	30.20	36.63	41.71	28.55
650	23.06	39.58	18.69	41.42	36.06	43.74	44.76	37.93
800	26.65	43.12	19.91	45.99	40.15	47.36	46.77	43.07
1000	26.64	46.68	20.41	48.56	41.24	50.23	46.98	46.51

Table 2. Stomatal conductance (mol $H_2Om^{-2}s^{-1}$) of 3 wheat mutants and their parent at PPFD of 1500 (µmol photons m^{-2} s^{-1}) as a function of CO_2 (µmol CO_2 mol^{-1} of the air) concentration at 20 and 30 °C.

CO_2 Concentration	BG48-3,1		BG48-3,7		BD29-3,9		Cham 1 (Parent)	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
100	0.501	0.622	0.365	0.562	0.615	0.554	0.415	0.502
200	0.482	0.556	0.353	0.463	0.592	0.526	0.393	0.465
400	0.540	0.642	0.338	0.545	0.596	0.564	0.494	0.416
500	0.494	0.644	0.414	0.544	0.655	0.582	0.466	0.494
650	0.501	0.665	0.426	0.682	0.624	0.604	0.328	0.532
800	0.513	0.593	0.434	0.682	0.616	0.606	0.298	0.524
1000	0.514	0.585	0.433	0.538	0.548	0.575	0.285	0.526

Table 3. Transpiration rate (mmol H_2O $m^{-2}s^{-1}$) of 3 wheat mutants and their parent at PPFD of 1500 (µmol photons m^{-2} s^{-1}) as a function of CO_2 (µmol CO_2 mol^{-1} of the air) concentration at 20 and 30 °C.

CO_2	BG48-3,1		BG48-3,7		BD29-3,9		Cham 1 (Parent)	
Concentration	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
100	5.16	8.84	4.02	8.16	5.87	8.29	4.46	7.02
200	5.07	8.08	3.91	7.40	5.76	7.89	4.30	6.57
400	5.46	8.87	3.75	7.79	5.75	8.12	4.94	6.19
500	4.97	8.67	4.56	7.96	6.29	8.24	4.86	6.50
650	5.11	9.25	4.66	9.16	5.91	8.56	3.80	7.21
800	5.22	8.44	4.72	9.15	5.79	8.47	3.47	6.97
1000	5.15	8.26	4.79	7.75	5.35	8.23	3.36	7.08

Table 4. Leaf intercellular CO_2 (µmol CO_2 mol air^{-1}) of 3 wheat mutants and their parent at PPFD of 1500 (µmol photons m^{-2} s^{-1}) as a function of CO_2 (µmol CO_2 mol^{-1} of the air) concentration at 20 and 30 °C.

CO_2	BG48-3,1		BG48-3,7		BD29-3,9		Cham 1 (Parent)	
Concentration	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
100	92.24	89.72	82.92	93.44	83.35	89.27	75.66	102.55
200	166.38	154.93	158.98	154.76	155.38	149.73	118.41	161.11
400	348.34	307.98	326.75	285.89	318.74	283.00	258.23	272.24
500	424.02	385.23	415.12	366.23	398.33	353.91	319.83	357.79
650	546.19	513.82	551.12	490.16	520.31	478.70	388.40	477.58
800	681.98	636.53	694.67	620.59	651.53	611.42	500.68	598.99
1000	879.15	814.91	889.11	774.23	830.37	786.74	686.26	778.27

Table 5. Photosynthetic activity (µmol $m^{-2}s^{-1}$) of 2 wheat mutants and their parent at reference CO_2 of 400 (µmol CO_2 mol air^{-1}) as a function of PPFD (µmol photons $m^{-2}s^{-1}$) at 20 and 30 °C.

PPFD	BG48-3,7		BD29-3,9		Cham 1 (Parent)	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
0	-4.87	-2.55	-3.69	-4.58	-2.59	-8.61
100	1.64	3.38	4.25	1.49	3.17	-4.23
250	5.00	11.27	9.03	9.32	12.87	4.02
500	8.72	22.96	15.01	17.58	22.13	11.28
1000	11.35	29.44	21.02	21.21	30.86	16.49
1500	10.22	20.71	25.03	21.45	34.35	16.33

Table 6. Stomatal conductance (mol $H_2Om^{-2}s^{-1}$) of 2 wheat mutants and their parent at reference CO_2 of 400 (µmol CO_2 mol air^{-1}) as a function of PPFD (µmol photons $m^{-2}s^{-1}$) at 20 and 30 °C.

PPFD	BG48-3,7		BD29-3,9		Cham 1 (Parent)	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
0	0.124	0.372	0.336	0.265	0.164	0.185
100	0.303	0.364	0.395	0.303	0.363	0.304
250	0.293	0.343	0.392	0.324	0.401	0.352
500	0.292	0.455	0.383	0.315	0.422	0.344
1000	0.355	0.566	0.444	0.284	0.345	0.393
1500	0.194	0.224	0.472	0.273	0.284	0.304

Table 7. Transpiration (mmol H_2O $m^{-2}s^{-1}$) of 3 wheat mutants and their parent at reference CO_2 of 400 (µmol CO_2 mol air^{-1}) as a function of PPFD (µmol photons $m^{-2}s^{-1}$) at 20 and 30 °C.

PPFD	BG48-3,7		BD29-3,9		Cham 1 (Parent)	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
0	1.06	4.06	2.25	3.09	1.10	1.90
100	2.20	4.30	2.76	3.85	2.35	2.92
250	2.25	4.43	2.78	3.94	2.73	3.55
500	2.43	5.95	2.97	4.25	3.12	3.68
1000	3.66	7.91	4.07	4.54	3.18	5.11
1500	2.51	4.19	4.84	4.75	3.04	4.76

Table 8. Leaf intercellular CO_2 (µmol CO_2 mol air^{-1}) of 3 wheat mutants and their parent at reference CO_2 of 400 (µmol CO_2 mol air^{-1}) as a function of PPFD (µmol photons $m^{-2}s^{-1}$) at (20 and 30 °C).

PPFD	BG48-3,7		BD29-3,9		Cham 1 (Parent)	
	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
0	466.23	405.06	419.03	422.93	429.95	480.25
100	382.12	371.23	369.47	382.56	373.47	421.25
250	359.39	325.86	343.98	339.74	323.08	369.38
500	335.09	281.15	312.28	286.28	271.96	327.86
1000	328.76	274.09	291.40	249.81	217.92	305.57
1500	299.59	224.00	292.38	233.45	176.23	293.14

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REFERENCES

Borlaug NE (2007). Sixty-two years of fighting hunger: personal recollections. *Euphytica*157: 287–297.

Borlaug NE, Dowswell CR (2005). Feeding a world of ten billion people: a 21st century challenge. In: R. Tuberosa, RL. Phillips, and M. Gale, eds., Proceedings of the International Congress in the Wake of the Double Helix: From the Green Revolution to the Gene Revolution, 27–31 May 2003, Bologna, Italy. Avenue Media, Bologna, Italy. pp. 3–23

Calderini DF, Reynolds MP, Slafer GA (1999). Genetic gains in wheat yield and main physiological changes associated with them during the 20th century. In: EH. Satorre, and. GA. Slafer, (eds)., "Wheat: Ecology and Physiology of Yield Determination", Food Product Press, New York, pp. 351-377.

Crafts-Brandner SJ, Law RD (2000). Effect of heat stress on the inhibition and recovery of the ribulose-1,5-bisphosphate carboxylase/oxygenase activation state. Planta 212: 67–74.

Feller U (2006). Stomatal opening at elevated temperature: an underestimated regulatory mechanism? *GEN. Appl. Plant Physiol.* Special issue: 19-31.

Jin SH, Jiang DA, Li XQ, Sun JW (2004) Characteristics of photosynthesis in rice plants transformed with an antisense Rubiscoactivase*gene*. *J. Zhejiang Univ. Sci*. 5(8):897-899

Haldimann P, Feller U (2005). Growth at moderately elevated temperature alters the physiological response of the photosynthetic apparatus to heat stress in pea (*Pisumsativum* L.) leaves. *Plant Cell Environ*. 28: 302–317.

Jensen RG (2000). Activation of Rubisco regulates photosynthesis at high temperature and CO_2. P. Natl. Acad. Sci. USA 97 (24): 12937-12938.

Law RD, Crafts-Brandner SJ (1999). Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Physiol*. 120: 173–181.

Kobza J, Edwards GE (1987) Influences of Leaf Temperature on Photosynthetic Carbon Metabolism in Wheat. Plant Physiol. 83: 69-74.

Kumar A, Li C, Portis AR (2009). Arabidopsis thaliana expressing a thermo-stable chimeric Rubiscoactivase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. *Photosynth. Res*. 100: 143–153.

Long SP, Zhu XG, Naidu SL, Ort DR (2006). Can improvement in photosynthesis increase crop yields? Plant Cell Environ. 29: 315–330.

Makino A, Sage RF (2007). Temperature response of photosynthesis in transgenic rice transformed with 'sense' or 'antisense' rbcS. *Plant Cell Physiol*. 48: 1472–1483.

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu XG, Price GD, Condon AG, Furbank RT (2011). Raising yield potential of wheat. II. Increasing

photosynthetic capacity and efficiency. *J. Exp. Bot.* 62: 453–467.

Parry MAJ, Madgwick PJ, Carvahlo JFC, Andralojc PJ (2007). Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. *J. Agr. Sci*. 145: 31–43.

Portis AR (2003).Rubiscoactivase - Rubisco's catalytic chaperone.*Photosynth. Res.* 75(1):11-27.

Raines CA (2006). Transgenic approaches to manipulate the environmental responses of the C3 carbon fixation cycle. *Plant Cell Environ*. 29: 331–339.

Reynolds MP, Ginkel MM, Ribaut JM (2000). Avenues for genetic modification of radiation use efficiency in wheat. *J. Exp. Bot.* 51, 459–473.

Sage RF, Way DA, Kubien DS (2008). Rubisco, Rubiscoactivase, and global climate change. *J. Exp. Bot.* 59 (7): 1581-1595.

Salvucci ME, Crafts-Brandner SJ (2004). Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiol. Plantarum* 120: 179–186.

Salvucci ME, DeRidder BP, Portis AR (2006). Effect of activase level and isoform on the thermotolerance of photosynthesis in Arabidopsis. *J. Exp. Bot.* 57, 3793–3799.

Sharkey TD, Badger MR, Caemmerer SV, Andrews TJ (2001). Increased heat sensitivity of photosynthesis in tobacco plants with reduced Rubiscoactivase. *Photosynth. Res.* 67: 147–156.

Yamori W, Suzuki K, Noguchi K, Nakai M, Terashima I (2006). Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. *Plant Cell Environ*. 29: 1659–1670.

Zhu XG, Long SP, Ort DR (2008). What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Curr. Opin. Biotech.* 19: 153–159.

Zhu, XG, Long SP, Ort DR (2010). Improving Photosynthetic Efficiency for Greater Yield. *Ann. Rev. Plant Biol*. 61: 235-261.