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EFFECT OF HEAT STRESS ON RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE IN RICE

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Key Word Index—*Oryza sativa*; Poaceae; heat stress; PAGE; ribulose 1,5-bisphosphate carboxylase; RLSU; RSSU; specific activity.

Abstract—Heat-induced changes of ribulose 1,5-bisphosphate carboxylase (RuBISCO, EC 4.1.1.39) at various times were estimated in tolerant (N 22) and sensitive (IR 8) cultivars of rice (*Oryza sativa* L.). At a temperature of 40°, specific activity of carboxylase and the titre of RuBISCO holoenzyme were increased or not affected, while at 45°, the specific activity and holoenzyme level were more stable in the tolerant cultivar than in the sensitive one. In both cultivars, a decline in activity and holoenzyme level with time was pronounced at 50°. RLSU was more affected by higher temperatures than RSSU in the tolerant cultivar. However, no such trend was noted in component proteins of the sensitive one.

INTRODUCTION

Temperature is one of the major ecological variables that determines the distribution of plants in nature [1]. The rapid decline of photosynthesis at high temperature is noticed in whole plants [2, 3] as well as in detached leaves [4]. Among the photosynthetic components, temperature most adversely affects photosystem II activity, non-cyclic photophosphorylation [2, 5–8], as well as RuBISCO, [1, 9–11]. The synthesis and maintenance of RuBISCO are governed in a complex manner by the cooperation of both the nuclear and chloroplast genomes [12, 13]. Owing to the complex mechanism, several regulation levels have been proposed to account for changes in RuBISCO activity.

Remarkable differences exist among rice cultivars in their temperature susceptibility [14]. The objective of the present investigation was to identify whether the specific activity of RuBISCO and its native protein and subunit levels change coordinately with thermal stress.

RESULTS AND DISCUSSION

The main feature of our investigation is the differential response of RuBISCO activity and its holoenzyme and subunit levels under various thermal stress in rice cultivars (N 22 and IR 8). The amount of total soluble protein decreased by 20 and 32% in N 22 and 24 and 38% in IR 8 after 240 min at 45° and 50°, respectively. No appreciable effect on the total soluble protein was observed at 40° in N 22 (Fig. 1a) while in IR 8, the soluble protein increased prominently (Fig. 1b).

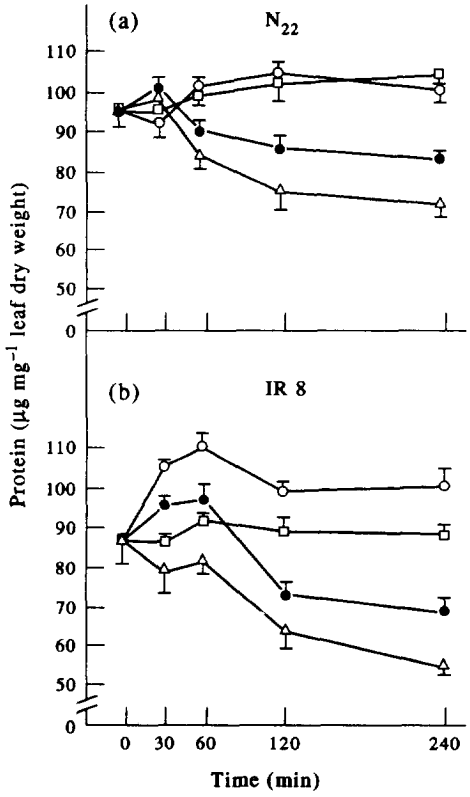

Fig. 1. Changes in total soluble protein in leaf extracts from 12 day-old-seedlings of rice (a) N 22 and (b) IR 8. Seedlings were exposed to 35° (□), 40° (○), 45° (●), 50° (△) for 4 hr and total soluble protein was measured as described in the Experimental. The symbols are means of three replicates ± SE.

The specific carboxylase activity of RuBISCO in the tolerant (N 22) and sensitive (IR 8) rice cultivars responds differently to various thermal stress regimes. Increased carboxylase activity of RuBISCO was observed at 40° in both long- and short-term stress in both cultivars (Fig. 2). However, response was better in N 22 (Fig. 2a and b). At 45°, RuBISCO activity showed greater short-term stability in IR 8 than in N 22 and it was increased by 39 and 62% in IR 8 and 10 and 45% in N 22 after 30 min and 1 hr, respectively. But during long stress (2 and 4 hr) N 22 showed greater stability. The activities were 64 and 29% higher in N 22, while in IR 8, the decline was 10 and 25% after 2 and 4 hr, respectively. Further increase of temperature to 50°, severely reduced the carboxylase activity of RuBISCO in both the genotypes indicating the inhibitory effect of such elevated temperatures to the enzyme. After 4 hr at 50°, the specific activities dropped by 54 and 65% in N 22 and IR 8, respectively. The results of experiments on moderate temperature stress (45°), suggest that thermostability of the carboxylating enzyme may be an important component of plant resistance to heat stress [15].

Greater stability of RuBISCO holoenzyme was observed in the tolerant genotype than in the sensitive one after 4 hr at 45°. However, after 1 hr at 50°, a deleterious effect was observed in both cultivars (Fig. 3a and b). Little increase in RuBISCO was observed at 40° in both cultivars.

The quantification of RuBISCO large (RLSU) and small (RSSU) subunit polypeptides using SDS–PAGE indicated that both subunits were affected by temperature stress (Fig. 4). In N 22, both subunits of RuBISCO increased up to 1 hr at 40° and 45°, beyond which there was a decline with increased duration of treatment

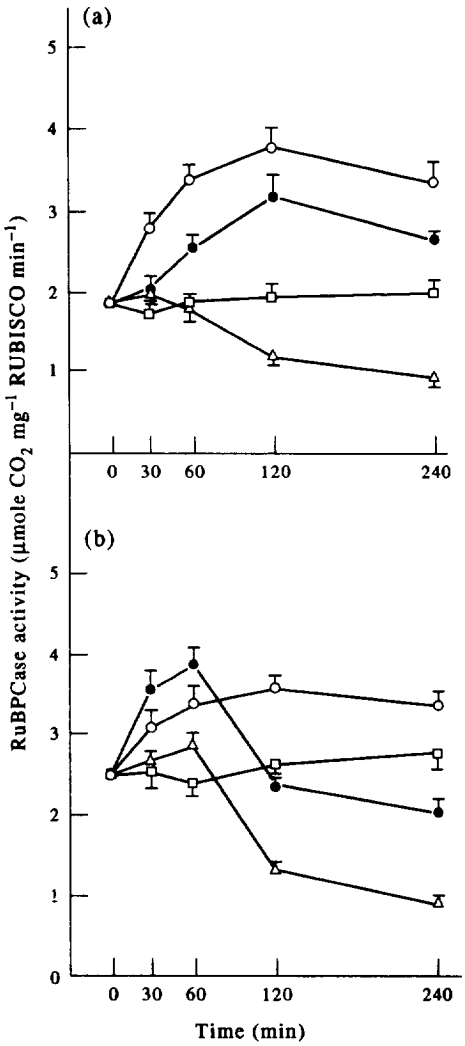

Fig. 2. Changes in specific activity of RuBISCO in leaf extracts from 12-day-old seedlings of rice (a) N 22 and (b) IR 8. Seedlings were exposed to 35° (□), 40° (○), 45° (●) or 50° (△) for 4 hr. RuBISCO activity was measured as described in the Experimental. The symbols are means of three replicates ± SE.

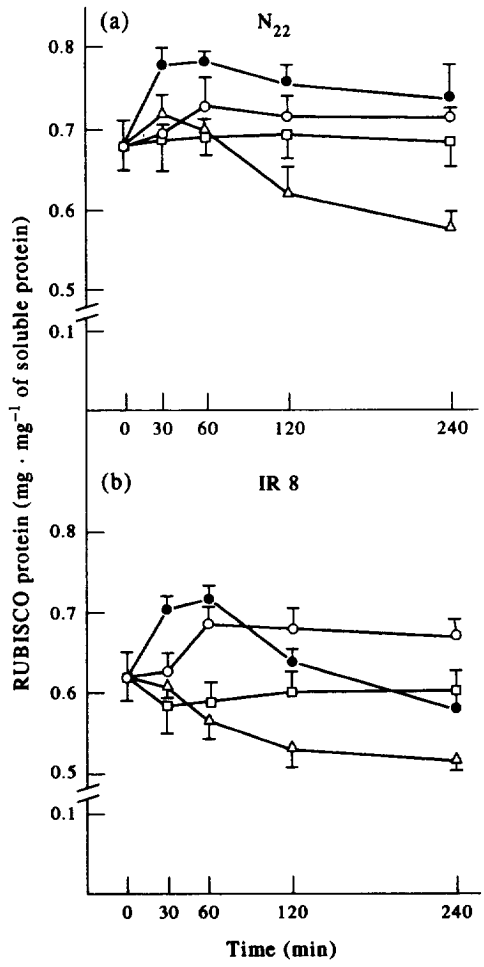

Fig. 3. Changes in RuBISCO holoenzyme protein in leaf extracts from 12-day-old seedlings of rice (a) N 22 and (b) IR 8. Seedlings were exposed to 35° (□), 40° (○), 45° (●) or 50° (△) for 4 hr. Quantification of RuBISCO was done from ND-PAGE densitometric scan as described in the Experimental. The symbols are means of three replicates ± SE.

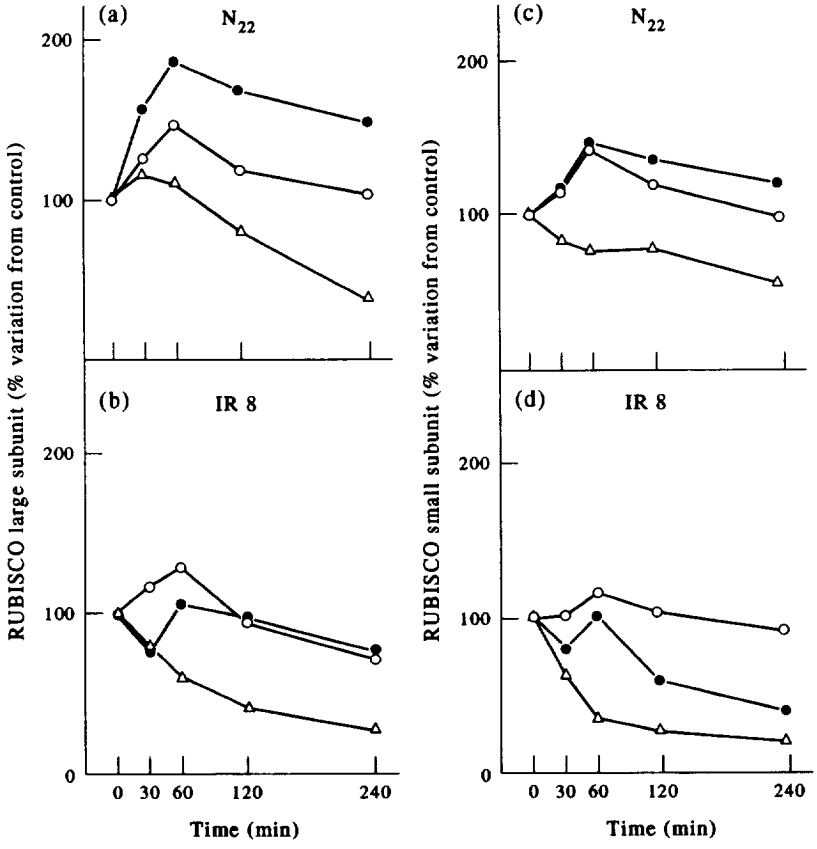

Fig. 4. Relative changes in RuBISCO large (a, b) and small (c, d) subunit proteins in leaf extracts from 12-day-old seedlings of N 22 (a, c) and IR 8 (b, d). Seedlings were exposed to 35° (□), 40° (○), 45° (●) or 50° (△) for 4 hr. Quantification of RuBISCO subunits was done from SDS–PAGE densitometric scans as described in the Experimental. Data are expressed in per cent of control values. Samples at 0 min of 35° were considered as controls whose absolute values were 43 μg/100 μg protein and 12.8 μg/100 μg protein for N 22 large and small subunits and 44 μg/100 μg protein and 14.4 μg/100 μg protein for IR 8 large and small subunits, respectively. The symbols are means of three individual experiments.

(Fig. 4a and c). The maximum increases of RLSU and RSSU were, respectively, about 50 and 40% at 40°, and 90 and 45% at 45° after 1 hr. At 50°, both subunits decreased gradually with time.

In IR 8, some increase was evident in RLSU only at 40° for the first 1 hr, thereafter it decreased significantly (Fig. 4b). Seedlings subjected to 45° showed a distinct decline in RSSU after 1 hr of treatment; however, in RLSU the decline was not as great (Fig. 4b and d). Exposure to 50° sharply reduced the levels of both subunits throughout the test period. So in the sensitive cultivar (IR 8), thermal stress (particularly of 45° and 50°) appears to be more harmful to both the component polypeptides of RuBISCO. However, RuBISCO protein and both subunits in the tolerant rice cultivar (N 22) show not only greater activity, but also more stability than in the sensitive one up to 45°.

Western blot analysis of RLSU confirmed that at 40° and 45° even after 4 hr, the RLSU level remained well above that of 35° in N 22, but in IR 8, its level declined after 1 hr of treatment. At 50°, comparatively faster degradation was noticed in IR 8 than in N 22.

Vierling and Key [16] have shown that synthesis of both subunits is similarly affected by heat treatment while the effect on mRNA is different. But the above results indicate that at 45°, the RLSU increased more prominently than the RSSU in the tolerant cultivar N 22. At this temperature after 4 hr, the effect of the RLSU was marginal, but the RSSU level declined sharply in the sensitive cultivar IR 8.

Within the photosynthetic apparatus, the light reaction, especially photosystem II, seems to be the most heat-sensitive function, whereas photosystem I activity, stromal enzymes, or chloroplast envelope are comparatively much more thermostable [1, 2, 8, 17, 18]. Ghosh *et al.* [15] suggested that the thermostability of carboxylating enzymes plays an important role in temperature regulation of the overall photosynthetic process as well as plant productivity. The present study also suggests that in rice, high temperature (45° and 50°) stress for prolonged periods inhibits RuBISCO activity, with greater effect in the sensitive cultivar than in the tolerant one. The changes in the quantity of RuBISCO per unit soluble protein in stressed leaf may be a result of differential

degradation of RuBISCO by protease during stress (chloroplasts contain protease capable of degrading RuBISCO [19]), loss of RuBISCO synthetic capabilities in the tolerant and sensitive cultivars, change in other soluble proteins and also increased synthesis of heat shock proteins.

EXPERIMENTAL

Materials. Seeds of *Oryza sativa* L. cv N 22 and IR 8 were obtained from International Rice Research Institute IRRI, Philippines, certified as 'thermal tolerant' and 'thermal sensitive', respectively. After surface sterilization with 0.1% $HgCl_2$ soln. the seeds were germinated in Petri dishes in H_2O in the dark for 4 days at 35°. The resulting seedlings were grown for 8 days in one-third strength Murashige and Skoog salt solution, pH 5.8, with their roots immersed in the nutrient soln, in a growth cabinet with a controlled day/night cycle of 10 hr/14 hr (light intensity 400 $\mu g\, m^{-2} s^{-1}$, temp. 35°/30° sec^{-1}).

Temperature treatments. Applied by placing the seedlings in a growth chamber controlled at 35° (control), 40°, 45° or 50° for 0.5, 1, 2, and 4 hr under controlled light intensity for 100 $\mu E\, m^{-2} s^{a-1}$. After treatments, the leaves were excised, cut into small pieces, frozen in liquid N_2 and stored at − 70° for subsequent processing.

Estimation of soluble protein. Leaf tissues (100 mg) were extracted with 1.5 ml of 10 mM K-Pi. (pH 7), passed through double-layered cheese cloth and centrifuged at 10 000 *g* for 10 min. The supernatant was analysed for total soluble protein using the method in ref. [20] with bovine serum albumin (fraction V, Sigma) as a standard.

Extraction of RuBISCO and assay of carboxylase activity. Leaf tissues (0.5 g) were rapidly ground in 4 ml of extraction buffer consisting of 0.1 M Tris–HCl, pH 7.8, 0.1 mM Na_2EDTA, 1.5% PVP, 5 mM 2-mercaptoethanol and 1 mM phenylmethylsulphonylfluoride. The homogenate was centrifuged at 15 850 *g* for 30 sec. RuBISCO was activated by incubating 0.9 ml of the extract supernatant with 0.1 ml of a preincubation mixture (0.1 M $NaHCO_3$ and 0.2 M $MgCl_2$) for 10 min. Carboxylase activity of RuBISCO was assayed according to ref. [21] by measuring ^{14}C-activity incorporated into acid-stable product. Activated enzyme (50 μl) was added to 450 μl of assay buffer consisting of 0.1 M Tris–HCl (pH 8), 20 mM $MgCl_2$, 1 mM EDTA, 13 mM $NaH^{14}CO_3$ (1 $\mu Ci.\, ml^{-1}$, sp. act. 59.1 $mCi\, mmol^{-1}$), and 0.5 mM ribulose bisphosphate.

Estimation of RuBISCO. The RuBISCO native protein was measured according to ref. [22] with slight modifications. The 15 850 *g* supernatant (1 ml) was lyophilized, redissolved in 0.5 ml protein resolving buffer [2.5 mM Tris–HCl, pH 8.5, 19.5 mM glycine, 10 mM 2-mercaptoethanol, 5% (w/v) sucrose with 0.1% Bromophenol Blue] followed by 6% non-denaturing polyacrylamide gel electrophoresis (ND-PAGE). The RuBISCO subunits were separated electrophoretically by horizontal 12.5% SDS-PAGE according to ref. [23] and quantification of the RuBISCO and its subunit was done by scanning the gel at 595 nm in an LKB 2202 Ultrascan, using spinach RuBISCO (Sigma) as an authentic standard.

Antisera preparation. Antisera of the large subunit of spinach RuBISCO were prepared according to ref. [15].

Electrotransfer and immunodetection of RLSU. Transfer of proteins into nitrocellulose membrane and immunodetection of RuBISCO large subunit by Western blotting were performed according to ref. [15].

REFERENCES

1. Berry, J. and Björkman, O. (1980) *Ann. Rev. Plant Physiol.* **31**, 491.
2. Al-Khatib, K. and Paulsen, G. M. (1989) *Plant Physiol.* **90**. 1041.
3. Harding, S. A., Guikema, J. A. and Paulsen, G. M. (1990) *Plant Physiol.* **92**, 648.
4. Grover, A., Sabat, S. C. and Mohanty, P. (1986) *Plant Cell Physiol.* **27**, 117.
5. Inoue, H., Kitamura, T. and Noguchi, M. (1987) *Plant Physiol.* **71**, 441.
6. Schreiber, U. and Armond, P. A. (1978) *Biochim. Biophys. Acta.* **502**, 138.
7. Thomas, P. G., Quinn, P. J. and Williams, W. P. (1986) *Planta* **167**, 133.
8. Havaux, M. (1992) *Plant Physiol.* **100**, 424.
9. Kobza, J. and Edwards, G. E. (1987) *Plant Physiol.* **83**, 69.
10. Leegood, R. C. (1985) *Photosynth. Res.* **6**, 247.
11. Monson, R. K., Stidham, M. A., William, G. J., Edwards, G. E. and Uribe, E. G. (1982) *Plant Physiol.* **69**, 921.
12. Highfield, P. E. and Ellis, R. J. (1978) *Nature* **271**, 420.
13. Miziorko, H. M. and Lorimer, G. H. (1983) *Ann. Rev. Biochem.* **52**, 507.
14. Hahn, M. and Walbot, V. (1989) *Plant Physiol.* **91**, 930.
15. Ghosh, S., Gepstein, S., Glick, B. R., Heikkila, J. J. and Dumbroff, E. B. (1989) *Plant Physiol.* **90**, 1298.
16. Vierling, E. and Key, J. K. (1985) *Plant Physiol.* **78**, 155.
17. Havaux, M., Greppin, H. and Strasser, R. J. (1991) *Planta* **186**, 88.
18. Weis, E. (1982) *Plant Physiol.* **70**, 1530.
19. Dalling, M. J. and Nettleton, A. M. (1986) *Chloroplast Senescence and Proteolytic Enzymes*, Vol. II, p. 125. CRC Press, Boca Raton, FL.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. H. (1951) *J. Biol. Chem.* **193**, 265.
21. Dann, M. S. and Pell, E. J. (1989) *Plant Physiol.* **91**, 427.
22. Leech, R. M., Leese, B. M. and Jellings, A. J. (1985) *Planta* **166**, 259.
23. Servaites, J. C., Torisky, R. S. and Chao, S. F. (1984) *Plant Science Letters* **35**, 115.