

Up-regulation of photosynthesis and sucrose-P synthase in rice under elevated carbon dioxide and temperature conditions

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ABSTRACT

Basmati rice (*Oryza sativa* L.) cultivars viz. PRH-10 (pusa rice hybrid-10) and PS-2 (Pusa Sugandh-2) were grown under two different day/night temperatures (31/24°C, 35/28°C) at ambient (370 µmol/mol) and elevated (550 µmol/mol) carbon dioxide (CO₂) concentration, respectively, to characterize how an increase in CO₂ and temperature affects rice photosynthesis and carbohydrate metabolism. At elevated CO₂, the photosynthetic rates increased under both the temperature regimes, compared with plants grown at ambient CO₂. The photosynthetic rate, sucrose-P synthase (SPS) activity and accumulation of soluble sugars and starch were higher in PRH-10 (pusa rice hybrid-10), compared to PS-2 (Pusa Sugandh-2). Elevated temperature decreased the photosynthetic rates both under ambient and elevated CO₂ conditions. The SPS (sucrose-P synthase) activity and the accumulation of soluble sugars and starch were enhanced at elevated CO₂ under both temperature regimes compared with plants grown at ambient CO₂. The up-regulation of SPS (sucrose-P synthase) under elevated CO₂ and temperature would be beneficial for growth and productivity of rice plants for the future climatic conditions.

Keywords: soluble sugars; starch; basmati rice

The environment is constantly changing as a result of human activities and the present challenge is to predict how agriculture will respond to the changing environment of tomorrow. Measurements of atmospheric carbon dioxide concentrations began at the South Pole, Antarctica, as early as in 1957, and at Mauna Loa (Hawaii) in March 1958, by using the analysis of air trapped in bubbles in ice cores. Mauna Loa studies observed an increase of 1.0 µmol/mol of CO₂ per year from 1958 to 1982 (Keeling et al. 1995). The current atmospheric concentration of CO₂ is 372 µmol/mol (Upreti et al. 2003), which is approximately 33% more compared to the industrial revolution. It is currently increasing at the rate of 1.5–1.8 µmol/mol per year. If this exponential rise of CO₂ in the atmosphere continues at the present rate, the concentration of CO₂ may be doubled by the end of 21st century (Houghton et al. 1996). Measurements in the Delhi region showed a similar increase in CO₂ concentration (Upreti et al. 2004).

A potential consequence of the rise in CO₂ concentration with respect to plant biology is its effect on plant process of photosynthesis – i.e. biological effect. The higher level of atmospheric CO₂ affects

C₃, C₄ and CAM plants differentially (Poorter 1993). CO₂ enhances the growth rate of almost all plants (Kimball 1983) but the enhancement was very significant in C₃ species. C₃ plants (rice, wheat, oil seeds, pulses etc.) respond to elevated CO₂ by reducing the oxygenase activity of RuBP carboxylase/oxygenase, enzyme resulting in the suppression of photorespiration (Long and Drake 1992). C₄ plants (maize, sorghum, sugarcane etc.) show little or no photosynthetic response to elevated CO₂, because they are CO₂ saturated and not competitively inhibited by O₂. The increase in CO₂ is expected to cause global warming by absorbing the long wave heat radiation from the earth surface and altering the precipitation (Moya et al. 1998) – i.e. climatic effect. According to global circulation models (GCMs), the temperature changes may not be uniform. At polar regions it may increase by 4–5°C and at equator by less than 1°C. From 1860 to 2000, the global average surface temperature increased by 0.6°C ± 0.2°C. The Third Assessment Report (TAR) of IPCC projects a global average warming of 1.4 to 5.8°C by the year 2100.

The warming that is predicted to occur will affect numerous physiological and morphological aspects

of plant development and would alter the source-sink relationship and biomass partitioning (Long and Woodward 1988). Models of the biochemistry of C_3 photosynthesis predict that acceleration of photosynthesis by CO_2 enhancement could be further increased with rise in temperature (Long 1991, Kirschbaum 1994) because elevated CO_2 levels will reduce photorespiratory losses. Idso and Idso (1994) showed that plant growth enhancement due to elevated CO_2 was highly significant at higher temperature. Moreover, the increased supply of assimilates caused by higher CO_2 could be combined with the increased sink metabolism resulting from the warming to produce larger plants with reduced feedback inhibition of photosynthesis and a carbohydrate flux that is independent of the pool sizes (Farrar and Williams 1991).

Therefore, an effort has been made in the present investigation to study the interactive effect of elevated CO_2 and temperature on rice varieties. Often, climate change studies are conducted under open top chamber or FACE conditions. In both these systems an inherent disadvantage is temperature that could be unstable due to increased carbon dioxide or chamber effects. However, in Phytotron, temperatures can be controlled, a simulation of climatic effect is possible and it is a better option for studies on climatic effects. In the present investigation, the temperature and CO_2 interaction studies were done using the Phytotron controlled growth chambers at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. The only disadvantage of this system is closed chamber.

MATERIAL AND METHODS

Basmati rice cultivars viz. Pusa RH-10 (PRH-10) and Pusa Sugandh-2 (PS-2) were used to study their response to the interaction of elevated CO_2 and temperature. In the Phytotron experiment, seeds were sown in trays containing 100% peat soil (decomposed coir pith). Deionized watering was done daily in the trays until the emergence of second leaf (i.e. up to 10 days of sowing). For the next 10 days half the strength of Hoagland's solution was given daily as nutrient solution. Seedlings were transplanted after 20 days to the pots of 20.32 cm (0.2032 m) diameter. Area inside the plant growth chamber was 3.34 m² and 45 pots were placed there. All the nutrients required by the rice crop were present in trace amounts in the peat soil. The peat soil also had a good water

holding capacity and it gave full support for the root spread. Full strength Hoagland's solution containing nutrients was given daily to the transplanted plants up to maturity.

Climatic conditions inside the plant growth chamber were similar to that of the natural environment. The RH maintained in the chamber was $70 \pm 2\%$ and the photoperiod of 16 h was given to the plants. The level of light was maintained with tube lights and incandescent bulbs.

Temperature and the carbon dioxide concentration were maintained in the chamber as follows:

- (a) ambient CO_2 conditions (370 ± 2 ppm) with temperature ($31/24 \pm 0.5^\circ C$);
- (b) ambient CO_2 conditions (370 ± 2 ppm) with temperature ($35/28 \pm 0.5^\circ C$);
- (c) elevated CO_2 conditions (550 ± 15 ppm) with temperature ($31/24 \pm 0.5^\circ C$);
- (d) elevated CO_2 conditions (550 ± 15 ppm) with temperature ($35/28 \pm 0.5^\circ C$).

The rate of photosynthesis was measured on intact leaves using a portable Infrared Gas Analyzer (IRGA, LI-COR-6200, Lincoln, Nebraska, USA). Fully expanded uppermost leaves of main shoot were used for sampling at the vegetative, heading and ripening stages of plant growth.

Uppermost fully expanded leaves were detached from five different plants from each treatment at different stages of growth before 12.00 a.m. at solar PPFD-500 $\mu mol/m^2/s$. The biochemical analysis was carried out using three replications for each observation. Samples were quickly immersed in ice bucket for the Sucrose Phosphate Synthase (SPS) extraction and activity estimation at $4^\circ C$. The enzyme was assayed and the activity was measured at vegetative, heading and ripening stages as per the method of Huber and Huber (1992). Leaf extracts were prepared by grinding the tissue frozen with liquid nitrogen in a standard buffer. The supernatant after centrifugation was desalted on a Sephadex G-25 column and assayed. Absorbance at 520 nm as determined by sucrose and sucrose-P production was expressed as μmol of sucrose synthesized/mg protein/min.

Sugars from fresh samples of leaves, stem and panicle were assayed according to the method of McCready et al. (1950) using arsenomolybdate reagent whereas starch content was determined by Anthrone method (Pucher et al. 1948, McCready et al. 1950) and expressed in mg/g dw. Statistical analysis of data was done following the method of analysis of variance (ANOVA) given by Panse and Sukhatme (1967). The critical difference (CD) values were calculated at 5% probability level.

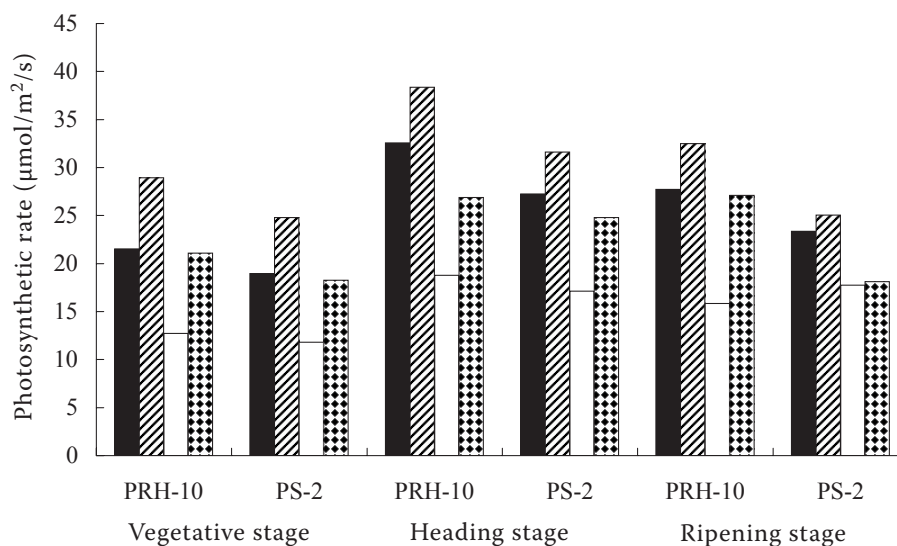


Figure 1. Interactive effect of elevated CO₂ and temperature on photosynthetic rate in rice varieties. The critical difference (CD) values were calculated at 5% probability level

Explanations to Figures 1–5: the bars from the left: ambient CO₂ + ambient temperature, elevated CO₂ + ambient temperature, ambient CO₂ + elevated temperature, elevated CO₂ + elevated temperature

RESULTS AND DISCUSSION

CO₂ enrichment brought about significant increase in photosynthetic rate and SPS activity at all the three plant growth stages. Similarly, a significant increase in the total sugar and starch content in the leaves, stem and panicle was observed in both the varieties under elevated CO₂ condition. Among the varieties PRH-10 had a higher SPS activity, sugar content and photosynthesis.

The CO₂ and temperature interaction on photosynthetic rate for both the varieties was signifi-

cantly different at the heading and ripening stages. At elevated CO₂, temperature-induced reduction in photosynthesis was decreased by about 11% at the heading stage of plant growth by PRH-10 as compared to the ambient CO₂ condition; in the case of PS-2, it was 8% (Figure 1).

The CO₂ and temperature interaction effect on the SPS activity in both rice cultivars is presented in Figure 2. At elevated CO₂, PRH-10 showed nearly about 25% reduction of the elevated temperature effect compared to the ambient situation. The SPS activity was significantly higher in the hybrid com-

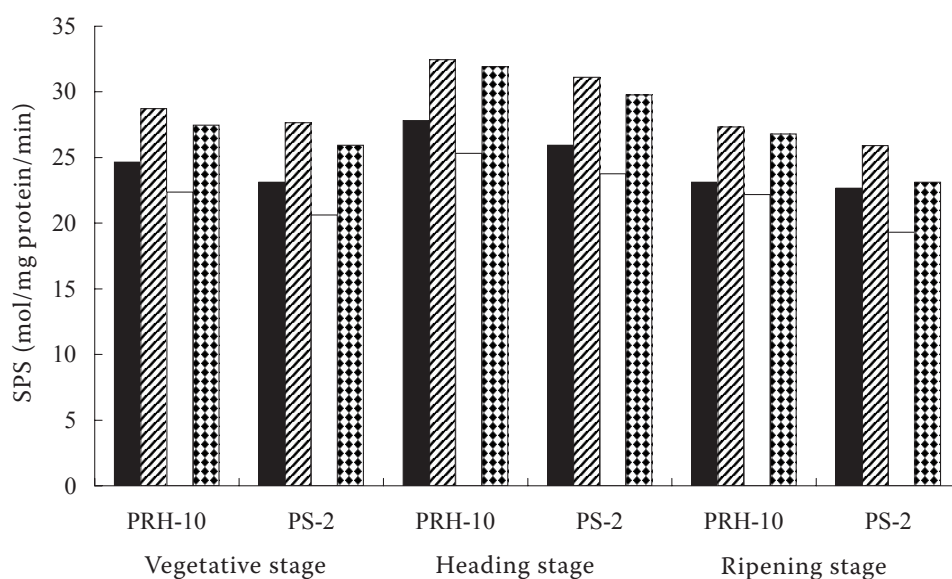


Figure 2. Interactive effect of elevated CO₂ and temperature on sucrose phosphate synthase activity in rice varieties. The critical difference (CD) values were calculated at 5% probability level. For explanations see Figure 1

Table 1. Interactive effect of elevated CO₂ and temperature on total sugar content in the leaves of rice varieties

	Vegetative stage		Heading stage		Ripening stage	
	PRH-10	PS-2	PRH-10	PS-2	PRH-10	PS-2
Ambient CO ₂ + ambient temperature	66.26	60.58	83.12	78.65	76.05	73.14
Elevated CO ₂ + ambient temperature	73.79	64.81	94.67	84.78	83.87	78.43
Ambient CO ₂ + elevated temperature	38.01	26.12	51.13	38.4	56.11	40.62
Elevated CO ₂ + elevated temperature	53.02	36.83	70.84	52.03	68.21	55.85
CD at 5%						
CO ₂ × <i>T</i>	NS		1.71		1.36	
CO ₂	1.25		1.21		0.96	
<i>T</i>	1.25		1.21		0.96	

The critical difference (CD) values were calculated at 5% probability level

pared to the PS-2 variety. The maximum amelioration effect of elevated CO₂ on the temperature induced reduction of SPS activity was observed at the heading stage of plant growth in both the varieties.

The interaction effect on total sugar content in the leaves, stem and panicle was significant both at the heading and ripening stage (Tables 1–3). In both varieties, the temperature-induced reduction in the total sugar content in different parts of the plant was less at elevated than at ambient CO₂. In the hybrid (PRH-10), high CO₂ treatment reduced the temperature-induced effect on total sugar content in the leaves by about 10–12% (Table 1), whereas it was 11% in the stem (Table 2) and 11% in the panicle (Table 3). As compared to PS-2, in PRH-10 the amelioration effect of elevated CO₂ was greater. Generally, the amelioration effect of

elevated CO₂ was more pronounced at vegetative and heading stages of plant growth. The same trend was observed in the case of starch content in different parts at different stages as is presented in (Figures 3–5). The CO₂ and temperature interaction on the starch content in the leaves and panicle was significant at all the stages.

Present investigation showed that at elevated CO₂ photosynthetic rate of the leaves increased. Baker and Allen (1993) also reported that doubling CO₂ from 330 to 660 μmol/mol increased rice canopy photosynthesis by 36%. The increase in photosynthesis due to elevation of CO₂ results from two properties of Rubisco (Long et al. 2004): (a) the K_m of the enzyme for CO₂ is close to the current atmospheric concentration, and elevated CO₂ increases the velocity of carboxylation; (b) CO₂

Table 2. Interactive effect of elevated CO₂ and temperature on total sugar content in the stem of rice varieties

	Vegetative stage		Heading stage		Ripening stage	
	PRH-10	PS-2	PRH-10	PS-2	PRH-10	PS-2
Ambient CO ₂ + ambient temperature	82.05	73.56	104.64	90.68	93.65	81.42
Elevated CO ₂ + ambient temperature	89.32	79.13	111.1	100.88	104.01	95.93
Ambient CO ₂ + elevated temperature	52.03	47.12	63.79	59.82	58.7	55
Elevated CO ₂ + elevated temperature	59.98	52.33	76.67	71.34	67.37	63.84
CD at 5%						
CO ₂ × <i>T</i>	NS		3.09		2.44	
CO ₂	1.25		2.18		1.72	
<i>T</i>	1.25		2.18		1.72	

The critical difference (CD) values were calculated at 5% probability level

Table 3. Interactive effect of elevated CO₂ and temperature on total sugar content in the panicle of rice varieties

	Heading stage		Ripening stage	
	PRH-10	PS-2	PRH-10	PS-2
Ambient CO ₂ + ambient temperature	139.71	130.77	205.21	183.82
Elevated CO ₂ + ambient temperature	154.13	145.59	231.91	209.04
Ambient CO ₂ + elevated temperature	112.54	107.71	142.2	133.41
Elevated CO ₂ + elevated temperature	127.9	120.76	160.2	154.65
CD at 5%				
CO ₂ × <i>T</i>	2.43		5.63	
CO ₂	1.13		2.32	
<i>T</i>	1.13		2.32	

The critical difference (CD) values were calculated at 5% probability level

competitively inhibits the oxygenation reaction, which produces glycolate leading to photorespiration. The latter effect is particularly important because it increases the efficiency of net CO₂ uptake by decreasing photorespiratory CO₂ loss and diverting ATP and NADP, which are generated in the light reactions, away from the photorespiratory metabolism to photosynthetic assimilation. Thus, the efficiency of net photosynthesis increases, regardless of other factors which limit gross photosynthetic rate (Long 1991, Long and Drake 1992). Thomas and Harvey (1983) however attributed this enhancement of photosynthesis to the addition of an extra layer of palisade cells in the leaf of soybean plants under elevated CO₂ condition.

In the present study, elevation of temperature with the two levels of CO₂ decreased the photo-

synthetic rate. This was probably due to the change in kinetic parameters of Rubisco and decreased solubility of CO₂ compared to O₂ that increased the photorespiration. Thus, higher temperature accelerates leaf maturation and senescence, decreasing the duration of active photosynthesis. Earlier, Lin et al. (1997) and Nakagawa et al. (1997) found that higher temperature stimulated single leaf photosynthesis of rice subjected to long-term CO₂ treatments during the vegetative stages. The temperature-induced reduction was ameliorated by CO₂ enrichment, since one of the major effects of elevated CO₂ on net photosynthesis is through the suppression of photorespiration. It could be expected that optimum temperature for photosynthesis shifts upwards as CO₂ increases (Horie et al. 2000). This type of interaction of temperature and CO₂ at leaf level photosynthesis was defined

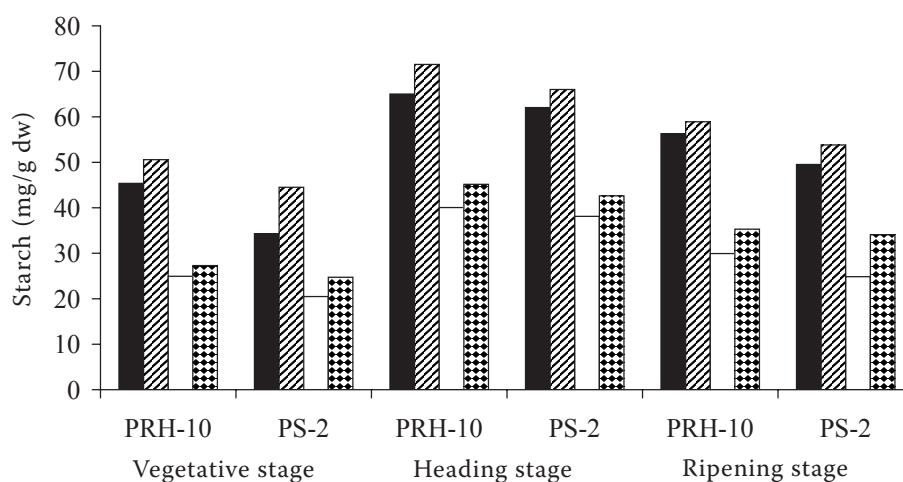


Figure 3. Interactive effect of elevated CO₂ and temperature on starch content in the leaves of rice varieties. The critical difference (CD) values were calculated at 5% probability level. For explanations see Figure 1

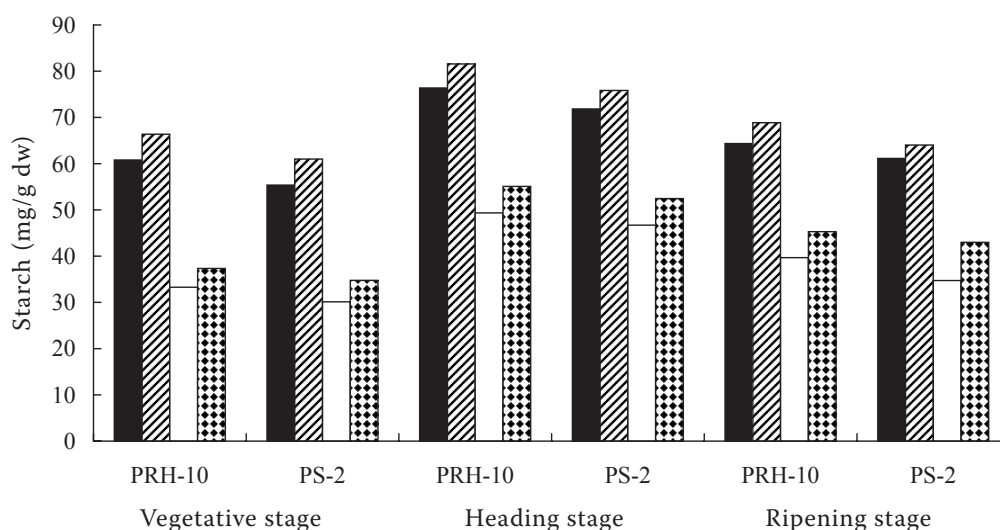


Figure 4. Interactive effect of elevated CO₂ and temperature on starch content in the stem of rice varieties. The critical difference (CD) values were calculated at 5% probability level. For explanations see Figure 1

by Long (1991) for some C₃ species and was confirmed in rice (Nakagawa et al. 1997).

CO₂ enrichment in rice enhanced the carbohydrate accumulation in different parts of the plant disturbing the partitioning balance. Increased carbohydrate production of elevated CO₂ was due to the stimulation of photosynthesis (Rogers et al. 2004). Ainsworth et al. (2002) observed a 45% increase in total non-structural carbohydrates (TNC) content in soybean. Prasad et al. (2004) reported in *Phaseolus* bean that elevated CO₂ upregulated activities of the carbohydrate metabolizing enzymes, sucrose phosphate synthase (SPS) and adenosine-5'-diphosphoglucose pyrophosphorylase (AGP), resulting in greater accumulation and export of

carbohydrates associated with photosynthetic activities, despite the decline in Rubisco activity and protein content.

An increase in the sucrose phosphate synthase (SPS) activity by about 29% at elevated CO₂ in rice was observed in the present study. The SPS activity increased as air temperature increased from 25°C to 34°C, whereas it decreased as the temperature rising from 35°C to 37°C. The increased SPS activity was due to the reallocation of nitrogen away from Rubisco and into SPS, leading to a greater sucrose synthesis and export of photosynthates from the leaves. These studies conclude that rice responds to increasing CO₂ concentrations by shifting the priority of allocation of carbohydrates

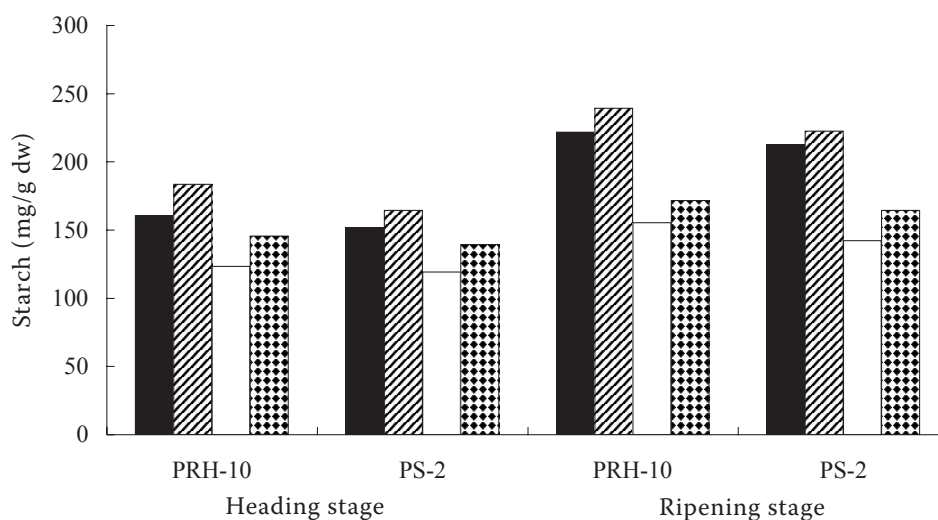


Figure 5. Interactive effect of elevated CO₂ and temperature on starch content in the panicle of rice varieties. The critical difference (CD) values were calculated at 5% probability level. For explanations see Figure 1

more towards export than carbohydrate storage, thus preventing the feedback inhibition (Rowland-Bamford et al. 1990). The trend in the present investigation was similar. Higher CO₂ enhanced the activity of sucrose phosphate synthase by 18% in PRH-10 and by 12% in PS-2, which led to higher sugar production in both varieties. Additional sugars produced at the initial stages increased the number of productive tillers as observed by Stitt (1991). This enhanced the production of panicles, which resulted in a higher number of grains. The higher amount of sugars produced at the heading stage was exported to these sinks, increasing the number of filled grains and grain mass, contributing thus to higher yield. The excess accumulation of carbohydrates in the leaves was accepted as one of the most important determinants for the development of new sinks (Stitt 1991, Sage 1994) that contribute to productivity.

Acknowledgements

The authors sincerely thank IARI for awarding the SRF, and Dr. S.R. Voleti for critically going through the manuscript.

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Received on November 30, 2008

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