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Impact of elevated CO_2 concentration on ultrastructure of pericarp and composition of grain in three *Triticum* species of different ploidy levels

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ABSTRACT

Triticum species of three ploidies were grown under ambient (375 μl/l) or elevated (FACE, 550 μl/l) CO_2 concentration [CO_2] to evaluate their response to CO_2 enrichment. The consistent effect of elevated CO_2 was an increase in concentration of starch and decrease in concentration of protein in the grain. Transmission electron micrographs revealed an increase in width and area of chloroplasts, and change in shape from elliptical in ambient to round in elevated [CO_2]. There was a corresponding increase in starch grain size and number in chloroplasts. The large starch grains distributed among thylakoids resulted in separation and distortion of internal membrane system in chloroplasts. The level of response was different in species of different ploidy levels. Maximum increase in starch concentration, and least decrease in protein concentration, was observed in *Triticum dicoccoides*, which also proved the most suitable species in terms of C:N ratio.

1. Introduction

The global atmospheric [CO_2] has increased from a pre-industrial value of 280 parts per million (ppm) to 370 ppm in 2005 (IPCC, 2007). CO_2 emissions have grown by almost 80% between 1870 and 2004, accounting for 77% of the total emissions of green house gases (GHG) in 2004. It is predicted that [CO_2] will reach twice the present value by the end of 21st century (Houghton et al., 1996). Within the global carbon cycle, photosynthesis is the key process which fixes inorganic carbon from the atmosphere to organic carbon in the living world. Effects of enhanced [CO_2] on physiology, growth and development have been studied in a number of plant species. Doubling of atmospheric [CO_2] from 330 ppm to 650 ppm may increase the productivity of a large number of C_3 crop plants on an average by 33% (Kimball and Isdo, 1983; Cure and Acock, 1986).

Wheat is one of the oldest and most widely cultivated cereals across the globe. Species of *Triticum* primarily exist in three ploidy levels—diploid, tetraploid and hexaploid. With an increase in ploidy, and a shift from wild to cultivated forms, the size of grain, leaf and duration of grain filling has increased manifold resulting in higher yield. However, the ability of wheat plant to compete and survive in natural conditions has declined with an increase in ploidy (Evans et al., 1975). Wheat yield has been projected to rise by up to 15% under enhanced [CO_2] (Amthor, 2001; Jablonski et al., 2002). Bloom et al. (2002) reported an increase in the leaf area and total plant biomass in wheat plants grown under enhanced [CO_2]. Apart from photosynthesis and respiration, studies have been carried out to observe the effect of elevated [CO_2] on morphology and ultrastructure of leaf in a wide range of plants (Vu et al., 1989; Griffin et al., 2001; Wang et al., 2004). Uprety et al. (2001) have reported a statistically significant increase in the length of epidermal cells, palisade cells and thickness of mesophyll in leaves of *Brassica juncea* exposed to elevated [CO_2]. Studies conducted on wheat by Seneweera and Conroy (2005) have reported an increase in the rate of leaf elongation and total leaf area by 32% and 18%, respectively, under enhanced [CO_2]. A significant acceleration in the rate of cell and chloroplast development in young wheat plants exposed to enhanced [CO_2] has been reported (Robertson and Leech, 1995). However, the effect of elevated CO_2 on ultrastructure of grain in wheat has not yet been investigated.

Several studies have reported that plants grown at elevated [CO_2] have higher accumulation of carbohydrates (Roumet et al., 1999; Seneweera and Conroy, 2005). Long et al. (2004) summarized the results obtained from experiments carried out in FACE facility on several species and concluded that elevated [CO_2] resulted in substantial increase in vegetative and reproductive biomass, decreased transpiration and reduced tissue quality in respect of protein and nitrogen content of leaves. A significant drop in protein concentration of major cereal crops such as rice, wheat, maize and barley by 10–15% has been reported (Maroco et al., 1999; Taub et al., 2008).

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Photosynthesis by developing caryopsis in wheat accounts for up to 42% of gross ear photosynthesis (Evans and Rawson, 1970). Separated grains contribute up to 40% of gross photosynthesis in wheat that is presumably derived from CO_2 fixation in the chlorophyll containing layers of pericarp (Watson and Duffus, 1988). Photosynthesis in the innermost cells of pericarp of developing wheat grain contributes to sucrose pool and hence starch synthesis in endosperm. It accounts for up to 2% of starch found in mature caryopsis (Watson and Duffus, 1988). Thus, caryopsis has the ability to directly fix atmospheric CO_2 and translocate the assimilates to endosperm/embryo. In spite of the significant contribution of the pericarp towards grain filling and quality, there has been no attempt to study the effect of enhanced [CO_2] on its ultrastructure.

The present study was carried out with an aim to observe changes induced by elevated [CO_2] in starch and protein concentration of the grain in correlation with ultrastructure of pericarp in three ploidy levels of wheat.

2. Materials and methods

2.1. Plant material

Grains of diploid wheat *Triticum monococcum*, tetraploid wheat *T. dicoccoides* and hexaploid wheat *T. aestivum* were procured from the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi (India).

2.2. Experimental design

T. monococcum, *T. dicoccoides* and *T. aestivum* were sown for three consecutive growing seasons from 2005 to 2008. The crop for each season was sown in the month of October and grains were harvested 10 days after anthesis. [CO_2] in the field ranged between 360 μl/l and 380 μl/l, 363 μl/l and 385 μl/l and 365 μl/l and 386 μl/l in 2005–2006, 2006–2007 and 2007–2008, respectively.

The trial consisted of two factors, factor one was [CO_2] (ambient 375 μl/l, and elevated 550 μl/l) and factor two was wheat species (*T. monococcum*, *T. dicoccoides* and *T. aestivum*). Treatment was given in a mid-FACE ring with [CO_2] of 550 μl/l. The field was prepared following the normal agricultural practices of ploughing, planking and hoeing. Plants were grown with 40 cm inter row spacing and 20 cm plant-to-plant space. Nitrogen, phosphorous and potassium fertilizers were applied at the rate of 60:60:40 kg per ha of the field. Control plants were grown following the same agricultural practices in an adjoining field wherein the only variable was [CO_2]. Mean ambient [CO_2] in the field was determined to be 375 μl/l.

2.3. Mid-free air CO_2 enrichment (FACE)

Wheat was sown in a mid-free air CO_2 enrichment (FACE) facility at the Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi within a crop rotation consisting of wheat and rice (Uprety et al., 2007). The mid-FACE ring was an octagonal shaped structure 8 m in diameter (Miglietta et al., 1997). The plenum was made of flexible irrigation pipes 20 cm in diameter with small vents through which CO_2 air mixture was released into the ring. Elevated [CO_2] of 550 μl/l was maintained in the FACE ring during daylight hours throughout the growing period of the plant and an infrared gas analyzer (IRGA) (Licor, 6200) was used to monitor the concentration of CO_2 in the ring. The valves of all eight nodes of the octagon were independently controlled with a computer-based system controller. CO_2 was injected from a CO_2–air mixing cylinder which was supplied CO_2 from 25 gas cylinder storage having manifold valves and flow meters. The fumigation of gas from the plenum was directed at the centre of the field 10–15 cm above the crop canopy level. This was done to reduce CO_2 gradient with depth and maintain a uniform concentration of gas throughout the ring. Height of the plenum was adjusted to the height of the canopy with the help of an adjustable stand. Wind direction and velocity were monitored, and the flow of CO_2 was released upwind of the plots and regulated according to an algorithm using CO_2 concentration and wind-speed as parameters.

2.4. Transmission electron microscopy (TEM)

For ultra structural investigations, grains from a middle spikelet of the central spike were harvested 10 days after anthesis from each of the six treatments. The grains were transversely cut into small pieces of 1 mm^3 and immediately fixed in Karnovsky's fixative for 18 h at 4 °C. After three washings in 0.1 M phosphate buffer for 15 min, each of these were post fixed in 1% osmium tetraoxide (0.2 M phosphate buffer) for 6 h at 4 °C. Subsequently, the grains were dehydrated by passing through a graded ethanol series at room temperature, embedded in araldite and then polymerized at 60 °C. Ultrathin sections of 90 nm, cut by glass knife, were stained with uranyl acetate and lead citrate. Sections were collected on copper grids and observed under a transmission electron microscope (Philips EM10) at 80 KV. Random samples of TEM micrographs were taken from at least five different grains of each ploidy for ambient and elevated [CO_2]. Length and width of chloroplasts were calculated on the basis of the scale of each micrograph and expressed in μm.

2.5. Estimation of starch and protein concentration

Freshly harvested grains were ground and homogenized in 80% ethanol, centrifuged at 3000 rpm for 10 min at room temperature. The pellet was rewashed with 80% ethanol till the washings stopped giving colour with anthrone reagent. To the supernatant 5 ml distilled water and 6.5 ml of 52% perchloric acid were added and centrifuged at 5000 rpm at 0 °C. Starch concentration was estimated by anthrone method using glucose as standard (Thimmaiah, 1999).

Freshly harvested grains were ground and homogenized in 0.1 M phosphate buffer (pH 7.2), filtered and centrifuged at 9000 rpm for 10 min at 4 °C. The supernatant was mixed with equal volume of 10% tri-chloro acetic acid and again centrifuged at 3300 rpm for 10 min at 4 °C. The pellet was washed with distilled water two or three times and then dissolved in 0.1N NaOH. Proteins were quantified by the protein dye binding method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

2.6. Statistical analysis

One-way analysis of variance was carried out for each parameter studied. Tukey's post hoc multiple mean comparison test was used to test for significant differences between treatments (5%). Univariate analysis was used to test significant differences in treatments, species and their interaction. All statistical analyses were performed with Statistical Package for Social Sciences version 10 (SPSS, Inc., Wacker Drive, Chicago, IL).

3. Results

The ultrastructure of chloroplasts in pericarp of wheat was significantly affected by enhanced [CO_2] in the three species with different ploidy levels. However, the response varied with the species. Pericarp chloroplasts of wheat plants grown at ambient [CO_2] were elliptical with well-stacked thylakoids and accumulated very little or no starch. Chloroplasts in plants grown at elevated [CO_2] were round and accumulated starch as irregularly shaped starch grains. At elevated [CO_2] the organization of the internal membrane system of chloroplasts was disrupted in all the