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Seed vigor of contrasting rice cultivars in response to elevated carbon dioxide

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ABSTRACT

Although a number of studies have shown that rising atmospheric carbon dioxide concentration, [CO_2], can differentially affect the growth and yield potential of rice (*Oryza sativa* L.) cultivars, there has been no attempt to determine if the response is associated with changes in seed vigor, an essential aspect of crop establishment. Because previous investigations have shown that [CO_2] can change the grain structure and quality of rice seed, we hypothesized that [CO_2] would decrease vigor via decreased germination rates. To test this hypothesis, we used an in situ, free-air CO_2 enrichment (FACE) system to assess seed quality in six rice cultivars that differed in their growth and reproductive response to rising [CO_2]. Elevated [CO_2] had no effect on seed hull thickness or seed specific gravity, but did significantly reduce total nitrogen and protein concentration for all cultivars. Despite the changes in grain physical and chemical traits associated with germination, no clear indication of quantitative effects of elevated [CO_2] on rice germination was found.

1. Introduction

Atmospheric carbon dioxide, CO_2, in addition to being a greenhouse gas, is also the source of carbon for most plant species. Its ongoing increase is likely to quantitatively and qualitatively alter a number of agriculturally relevant crop species. Among such species, rice (*Oryza sativa* L.), a C_3 crop, is recognized as a significant source of calories globally (IRRI, 2002); consequently, there has been ongoing research to determine how rising CO_2 levels will effect rice production and nutritional quality (Baker, 2004; Hatfield et al., 2011; Shimono and Bunce, 2009; Shimono et al., 2009; Ziska et al., 1997).

To date, a number of studies have shown that elevated [CO_2] differentially affects the growth and yield response of rice cultivars (Seneweera et al., 2001; Zhu et al., 2012). This variation is evident in a number of physiological responses; from the cellular to the whole plant level including changes in grain quality (Madan et al., 2012; Myers et al., 2014; Seneweera and Conroy, 1997; Shimono et al., 2009).

Although [CO_2] effects on grain quality, including physical and chemical characteristics, have been reported, to date, no information is available with respect to whether [CO_2] alters seed germination and/or seedling vigor, and whether such variation is the basis for cultivar variation in growth or yield. Yet, [CO_2] induced changes in seedling traits would be of obvious importance for seed establishment and crop-weed competition (Rajjou et al., 2012).

In this study, we hypothesized that [CO_2] enrichment may negatively affect seed vigor; including seed germination rate, germination energy and seedling growth characteristics (ISTA, 1996). To test this hypothesis, six rice cultivars having contrasting degrees of growth and yield stimulation in response to elevated [CO_2] were examined, and two of these cultivars were used for additional experimentation and qualitative analysis. To our knowledge, this is the first field study to specifically examine the role of elevated [CO_2] on seed vigor in cultivated rice.

2. Materials and methods

2.1. Experimental site and growth condition

The study was conducted at the FACE (Free-air carbon dioxide enrichment) platform located in Zongcun village (32°35′5″N,

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119°42′0″E), Jiangdu county, Jiangsu province. This province is located in the Yangtze River Delta region, experiences a subtropical monsoon climate and is typical for rice–wheat rotation. The soil is classified as a Shajiang-Aquic Cambiosol with a sandy loam texture. Soil properties at the experimental site are as follows: bulk density 1.16 g cm^{-3}, soil organic carbon 18.4 g kg^{-1}, total nitrogen 1.45 g kg^{-1}, total phosphorous 0.63 g kg^{-1}, total potassium 14.0 g kg^{-1}, available phosphorous 10.1 mg kg^{-1}, available potassium 70.5 mg kg^{-1}, and pH 6.8 (Xie et al., 2012).

The FACE operation for this location has been described previously (Okada et al., 2001). Briefly, it consists of three identical 14 m diameter octagonal rings where [CO_2] concentration is increased to 200 μmol mol^{-1} above the ambient concentration (Elevated), and three additional rings that do not receive supplemental CO_2 (Ambient). Rings are separated by 90 m to prevent CO_2 contamination between plots. In the elevated plots, pure CO_2 gas was released 24 h day^{-1} from peripheral emission tubes set at 50 cm above the crop canopy. Elevated CO_2 concentrations were achieved within 80% of the set point >90% of the time for each year. Daytime [CO_2] concentration in the elevated rings averaged over the growing season was 571 and 588 μmol mol^{-1} for 2012 and 2013, respectively. All other environmental conditions were consistent with cultural agronomic practices for this region.

Six rice cultivars [Koshihikari (KH), Yangdao 6Hao (Y6), IIY084 (II 84), Zhonghua14 (Z14), Wuyunjing21 (W21), Takanari (TK)] that are known to differ in their relative stimulation to [CO_2] were manually transplanted at a density of 3 seedlings a hill and 24 hills per m^2 for all six rings. Average (24 h) air temperature was 24.4 and 24.8 °C and seasonal mean relative humidity 92.9% and 91.9% for 2012 and 2013, respectively (Fig. 1). Total nitrogen (N, at 22.5 g N m^{-2} each season) was applied as a basal dressing (40% of the seasonal total), 1 day prior to transplanting and as a top dressing at early tillering (30% of the seasonal total) and at panicle initiation (PI) stage (30% of the seasonal total). Phosphorous (P) and potassium (K) were applied as a compound fertilizer at 9 g P_2O_5 m^{-2} and 9 g K_2O m^{-2}; both P and K were applied as a basal dressing 1 day before transplanting.

2.2. Sampling and measurements

Seed yield was determined by cultivar and [CO_2] treatment for all plants within a 2 m^2 area (excluding border hills). Grain weight was adjusted to a 14% moisture content (Yang et al., 2006a) and 1000-grain weight determined. Seed specific gravity was calculated as:

$$X = \frac{W}{(V_2 - V_1)}$$

where X is specific gravity (g ml^{-1}), W is the weight of clean ripened seeds (g), and V_1 and V_2 are the change in displaced volume in milliliters (RAST, 1995).

Hull thickness was determined for 50 full grains (RAST, 1995) using an electronic vernier caliper (GB/T14899-1994), and defined as:

$$Y = \sum (L_2 - L_1)/2/50$$

where Y is hull thickness (mm), L_2 and L_1 are the diameter (mm) of full grains and brown seed, respectively.

Germination rate was determined following the ISTA (1996) guidelines. Briefly, one hundred ripened grains were disinfected by 0.15% formalin for 20 min, and then soaked for 24 h. Seeds from each replication and treatment were placed in a culture dish (15 cm diameter) which contained moistened filter paper and kept at 28 °C. Seed germination was recorded every 24 h over a seven day period to determine germination rate. Seed was considered germinated

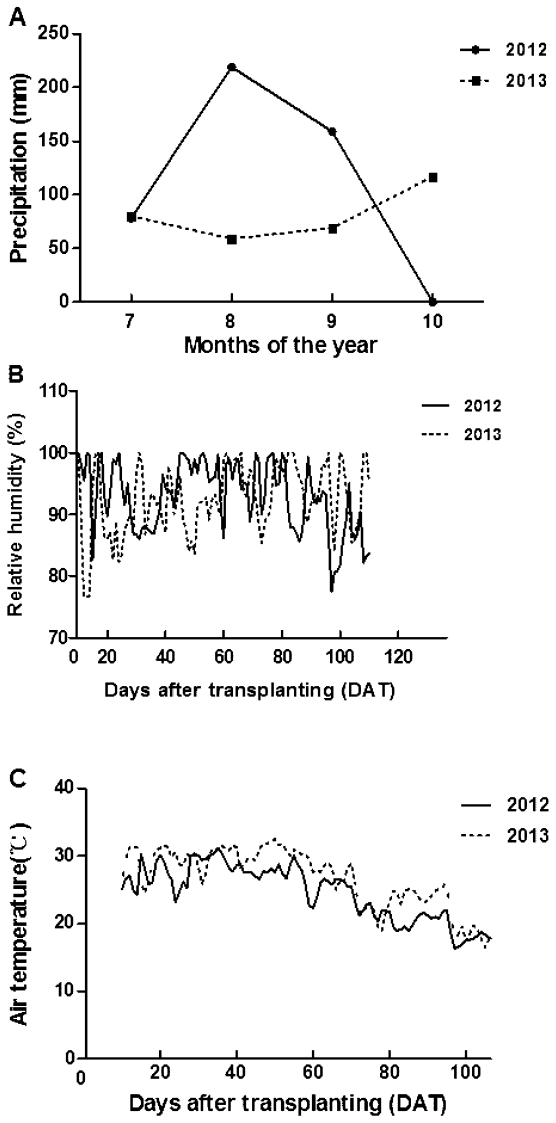

Fig. 1. Seasonal changes in (A) precipitation, (B) relative humidity, (C) air temperature during the growing season for the 2012 and 2013 growing season.

when the testa was broken and the radical was clearly visible. Germination energy was calculated as:

$$GI = \sum \left(\frac{Gt}{t}\right)$$

where GI is germination energy, Gt is germinated seed number on day t, t is the corresponding germinating day, respectively. Following germination, seedling fresh weight and dry weight were also for all healthy seedlings to record growth status.

Electrolyte leakage conductivity (CL) was quantified by using a DDS-12A conductometer for all treated rice seed. CL was calculated as:

$$CL = \frac{(C_2 - C_1)}{W}$$

where CL is the conductivity [μs $(cm\ g)^{-1}$], W is the weight of thirty completely cleaned fully mature grains (g), the seeds were soaked in 30 ml of distilled water for 24 h, C_1 (μs cm^{-1}) and C_2 (μs cm^{-1}) are the conductivity determined at time zero (C_1) and time after 24 h (C_2), respectively.

At the end of 24 h, the seed lixivium was tested for soluble sugars (soluble sugar in seed leakage, SSL) and soluble protein (soluble