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Research Article

Impact of elevated carbon dioxide and different nitrogen doses on grain quality of rice (*Oryza sativa* L.)

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Abstract

Rising atmospheric carbon dioxide concentration [CO_2] has the potential to enhance the growth and yield parameters of rice (*Oryza sativa* L.), but less is known regarding the impact of elevated CO_2 on grain quality of rice, especially under different soil nitrogen (N) levels. In order to investigate the same, a free-air CO_2 enrichment (FACE) experiment was conducted which showed a serious deterioration of processing quality. Results showed a significant reduction in milled rice percentage (MRP) (-1.7%) and head rice percentage (HRP) (-15.4 %) while brown rice percentage (BRP) increased (+3.2 %) under elevated CO_2 (EC) condition. Amylose content (AC) showed a significant increase (+14.2%) under EC. Gelatinization Temperature (GT) was inversely correlated to ASV which also get increased (-0.4 ASV). Gel Consistency (GC) increased (+10 to 11%) under FACE. These changes in grain quality revealed that hardness of grain increased with EC. Further nutritional quality also get negatively influenced by EC due to reduced protein content (-10%), N (-9.6%), P (-17.8%), K (-7.2%), Iron (-5.8%) and Zinc (-7.4%); contrary, protein yield exhibited an opposite trend showing a significant increase (+11%) under FACE. Overall, N supply had less but significant influence on rice grain quality showing trends of better appearance quality for N2 grains as compared with N0 or N1-grains. The adequate use of N fertilizer application may significantly contribute to counter the adverse effect of rising atmospheric [CO_2] on rice grain quality.

Keywords: Climate change, Elevated CO_2, Free-air CO_2 enrichment (FACE), Grain quality, Rice (*Oryza sativa* L.), Nitrogen levels

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Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world and is the staple food for nearly half of the world's population [1]. Global climate change is one of the biggest challenges of the twenty first century [2]. Improvements in rice productivity and quality must be achieved under projected climate change conditions, which will have significant impacts on crop yield and quality [3]. Rising atmospheric [CO_2] and related CO_2 fertilization effect may have regulating effects on numerous aspects of growth, plant physiology and biochemistry in various crops [4-7]. This could have significant implications for public health and nutritional securities of nation. Elevated [CO_2] will have positive effects on biomass and grain yield by promoting photosynthesis [7], whereas negative effects are expected on grain quality. Grain quality get affected in terms of nutrition (protein content, N, P, K, Fe and Zn) [8], appearance (grain length, width, grain chalkiness and hardness) and cooking properties (Amylose content, Gelatinization Temperature, Gel Consistency) [9, 10]. Rice grains produced under elevated CO_2 exhibited lower mineral (viz., N, P, Zn and Cu) and protein contents, while amylose content was higher than in those grown under ambient CO_2 [11, 12], produced harder grains grown under elevated CO_2. Some experiment showed that FACE decreased the protein content, increased maximum viscosity, but did not change amylose content [13]. Further it has been reported that only N concentration in rice grains was negatively affected by CO_2 enrichment, with other macro- (viz., P, K, Mg, S) and micronutrient contents (viz., Zn, Mn, Fe, B, Mo) remaining unaffected [14]. Further Rice quality is determined not only by its nutritional value but also by its appearance, milling performance, and cooking characteristics. Although these quality attributes differ, depends upon its end use but appearance, milling and the cooking quality of the rice are of prime concern among consumers [9, 11, 13, 15]. Their findings indicate that detailed knowledge of effect of elevated CO_2 on rice grain quality response to elevated CO_2 is still lacking and inadequate to the date.

Apart from that, several other factors may play significant role in achieving higher yields under elevated CO_2 conditions. Nitrogen (N) is the most important element that plants absorb from the soil and contributes to the plant growth, yield as well as quality and may play an important role in the response of plant to CO_2 [16-18]. Less report is available that if and how the rice quality responses to rising CO_2 and how it will be moderated by N supply.

As rice supplies increase to meet demand, and as incomes rise throughout the region, rice consumers become increasingly concerned with the chemical and physical characteristics of the rice grains that they buy. However to the date, there are fewer studies on the effects of elevated CO_2 on grain quality compared to growth and grain yield. Therefore, determining the effects of elevated CO_2 on these attributes would be extremely valuable to assess consumer perceptions of rice. The objective of this study was to determine the impacts of elevated CO_2 projected to occur in the near future on milling suitability, grain appearance, nutritional quality and physiochemical traits influencing rice eating quality under FACE with varying levels of soil nitrogen supply to the crop.

Materials and Methods

Study site, treatments and crop management

The experiment was carried out at the Climate Change Research Facility site of the Centre for Environment Science and Climate Resilient Agriculture (CESCRA), Indian Agricultural Research Institute, New Delhi, India (28°35' N and 77°12' E). The promising rice variety 'Pusa 44' was grown both under ambient CO_2 condition (AC) and elevated CO_2 (EC) conditions during *kharif* season of 2013-14 using Free Air CO2 Enrichment (FACE) facility established at the centre (**Figure 1**) [19]. In brief the FACE facilty consist an octagonal ring of 6 meter diameter which was used to inject pure CO_2 throughout the growing season and CO_2 was set at 550±20 ppm (EC) at crop canopy level using SCADA software based FACE facility. The plants grown under ambient CO_2 level (390±20 ppm, AC) served as control. Non drained pots were used for rice cultivation. Rice transplanting was done with two hills per pot with plant to plant distance of 20 cm. The three N levels were- without external nitrogen input (N0), 0.8 g N pot-1 (N1) and 1.0 g N pot-1 (N2), which were equivalent to Control, 100% and 125% recommended dose of N respectively. Recommended dose of N was taken as 120 kg N ha^{-1}. In total there were 6 treatments with 3 replications each. Phosphorus and potassium were applied as basal dose @ 60 and 40 kg $ha^{-1,}$ respectively. One third of N dose was applied as basal and remaining in 2-3 splits at vegetative and reproductive growth stages. Crops were maintained free from weeds and pests. Crops were harvested at maturity (about 13-14 % grain moisture).

Figure 1 Fee Air Carbon dioxide Enrichment facility at IARI Farm, New Delhi

Determination of grain quality characters

In the year 2013, rice grains for yield and quality analysis were obtained from panicles of main tiller of each pot. After harvest, the grains were threshed carefully and filled grains were selected manually, these filled grains were dried to <13% moisture content. The evaluation of rice processing quality, namely brown rice percentage (BRP), milled rice percentage (MRP) and head rice percentage (HRP), following the standard methods [9]. BRP is defined as the weight percentage of brown rice obtained from a sample of rough rice, while MRP is expressed as the weight percentage of milled rice obtained from a sample of rough rice. Head rice refers to milled rice with a length greater than or equal to three-quarters of the whole kernel, and HRP is expressed as the weight percentage of head rice obtained from a sample of rough rice. The appearance quality characteristic of brown rice viz., grain morphology includes grain length, grain width and the ratio of grain length to width, were measured by using standard graph paper method followed from National DUS Test Guidelines (2007) for rice [20]. The oven-dried samples were ground with a stainless steel grinder with a 100-mesh sieve in order to further prepare them for nutritional and cooking qualities. Total N in sample was estimated using nitrogen analyzer, potassium by flame photometer, phosphorus in UV Visible spectrophotometer following method described by Jackson [21]. Micronutrients, viz., Iron (Fe) and Zinc (Zn) were quantified using atomic absorption spectrometer instrument. Protein content (PC) was also determined by using Folin-Ciocalteau reagent [22]. Further Amylose content (AC) was quantified by following Juliano protocol [23] and Gelatinization Temperature (GT) via Alkaline Spreading Value [24]. Gel consistency (GC), an important eating quality parameter, was measured by using rice paste, digested by 0.2 N KOH [25] solutions in culture tube and final reading were taken after 30 minutes at room temperature.

Statistical analysis

Data were analyzed with the statistical package SPSS10.0 and EXCEL'2010. The experimental design was completely randomized design (CRD). Data were statistically analyzed by the ANOVA procedures to determine the main and interactive effects of the two factors of CO_2 and N. If the hypothesis of equal means has been rejected by the ANOVA test, the Fisher LSD procedures were employed to distinguish among treatment means. Unless indicated otherwise, differences were considered significant at $P<0.05$.

Results and Discussion

Processing quality

The processing quality is one of the basic traits as it is directly related to the market value of rice and mainly deals with final milled rice grain yield. In the present study, the processing quality was evaluated by using three parameters, brown rice percentage (BRP), Milled rice percentage (MRP) and Head rice percentage (HRP). In the present study results showed a significant increase in BRP both under elevated CO_2 condition and N doses. BRP showed a maximum of 3.2 % increase under elevated CO_2 condition with recommended N dose (N1) (**Figure 2a**). MRP and HRP both responded negatively and significantly got reduced under elevated CO_2 condition over ambient. But across both CO_2 levels, MRP and HRP increased significantly with increased N doses and found to be maximum under N2 treatment. The integrated MRP and HRP averaged over all N dose levels found to be reduced by 1.7 % and 15.4 % respectively (Figure 2b, c). The decrease in milling traits (MRP and HRP) pointed towards a decreased milling suitability under elevated CO_2 condition and similar results has been reported by many other researchers [9, 13 and 26]. Lower MRP indicates higher bran removal from the brown rice and more damaged and broken grains during milling process which may be due to lower grain hardness under FACE facility attributed towards lower HRP. An overall decrease of 15.45 % in the HRP is a considerable factor which can influence the market value in coming future, as whole grains are preferred more over the broken ones.

Brown rice, Milled rice and Head rice Yields were also found to have a significant increase under elevated CO_2 condition over ambient, regardless of N application levels. In both the CO_2 levels RR, BR, MR and HR yields were also increased significantly with increased N doses and their maximum value reported to be 71.9 g pot^{-1}, 59.8 g pot^{-1}, 49.9 gram pot $^{-1}$ and 35.0 g pot^{-1} respectively under FACE with N2 treatment (**Table 1**).The results showed a non-significant interaction between CO_2 and N on these processing quality assessment parameters.

Appearance quality

Appearance quality mainly depends on grain length, width and their ratios in the case of brown rice. Under elevated CO_2 condition it was found that grain length and width significantly get increased but the change was very small and similar was in the case of N treatments. On averaging for the N treatment, an increase of 1.23 % and 1.05% for grain length and grain width respectively has been observed under elevated CO_2 condition over ambient (**Figure 3** a, b). Maximum value for grain length (6.35 mm) and width (1.93 mm) were found under elevated CO_2 condition with N2 treatment. Although the two dimensions of grain increased significantly under elevated CO_2 condition but they do not let any change in the length to width ratio of rice grain (Figure 3c). Similar result was reported in an experiment [26] and supported increased grain length (1.65 %) and width (1.86 %) under elevated CO_2 condition, related to increased grain biomass.

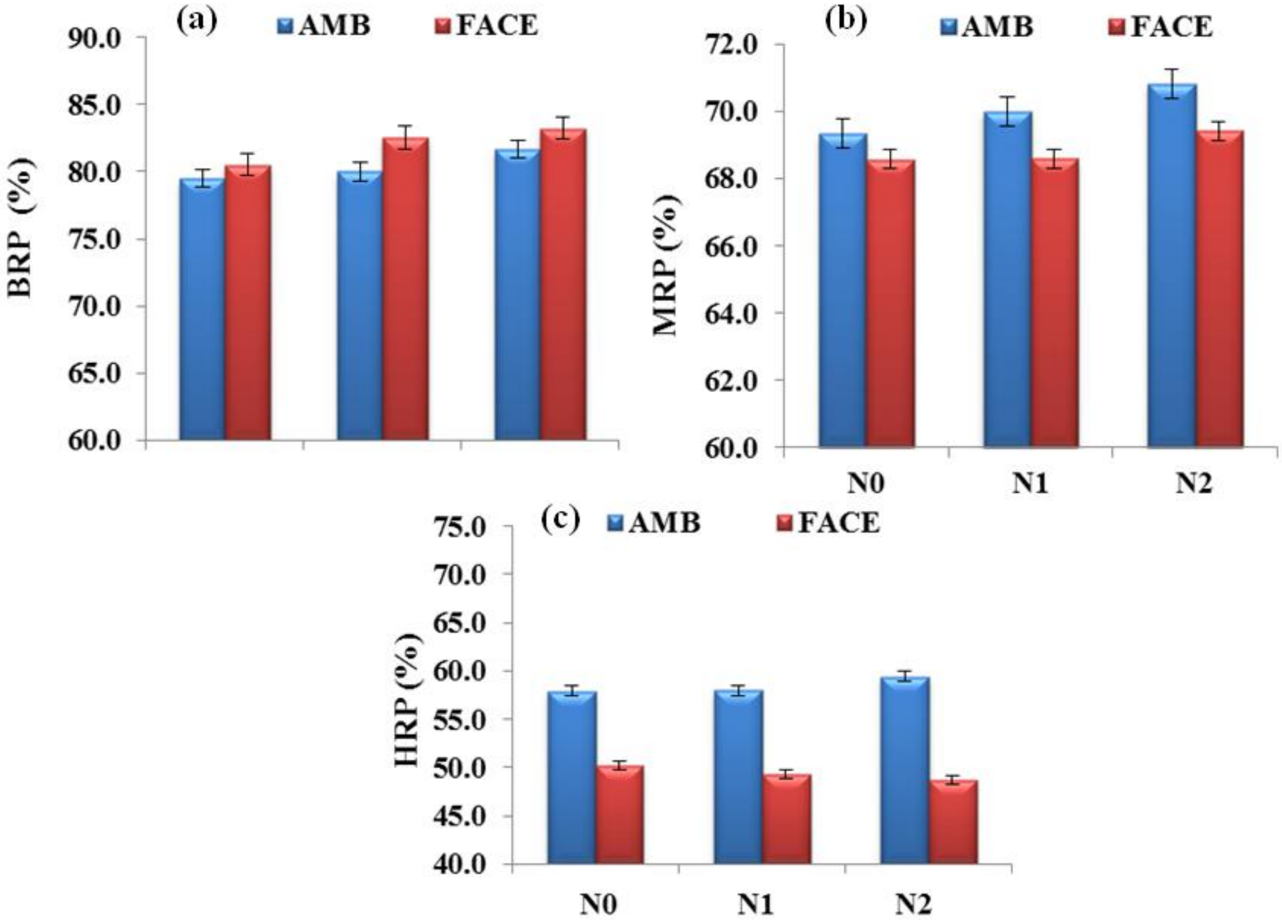

Figure 2 Impact of elevated CO2 and N levels on processing quality parameters of rice grain- (a) Brown rice percentage (BRP), (b) milling rice percentage (MRP) and (c) head rice percentage (HRP).

Table 1 Impact of elevated CO$_2$ and N doses on processing quality parameters of milled rice

N Treatments	CO$_2$ Treatments	RR Yield (g pot^{-1})	BR Yield (g pot^{-1})	MR Yield (g pot^{-1})	HR Yield (g pot^{-1})
N0	AMB	43.7	34.7	30.3	25.3
	FACE	51.2	41.2	35.1	25.7
	% Change	17.2	18.6	15.9	1.6
N1	AMB	54.5	43.6	38.2	31.6
	FACE	68.4	56.5	46.9	33.8
	% Change	25.5	29.5	23.0	6.8
N2	AMB	57.0	46.6	40.4	33.9
	FACE	71.9	59.8	49.9	35.0
	% Change	26.1	28.6	23.6	3.4
ANOVA	N	**	**	**	**
(P= 0.05)	CO2	**	**	**	**
	N x CO2	NS	NS	NS	NS

Probability levels are indicated by NS, * and ** for ‘not significant’, 0.05, and 0.01, respectively. (AMB - 550±20 ppm, EC and FACE - 390±20 ppm, AC)

Cooking quality

Consumer’s choice of rice varieties are largely based on grain and cooking qualities. From consumption point of view boiled rice is preferred the most in Asian continent. So it is very important aspect of grain quality which also determines the economic value of rice. Cooking / eating quality is determined by three primary physico-chemical characteristics of starch that are Amylose content (AC), Gel consistency (GC) and gelatinization temperature (GT).

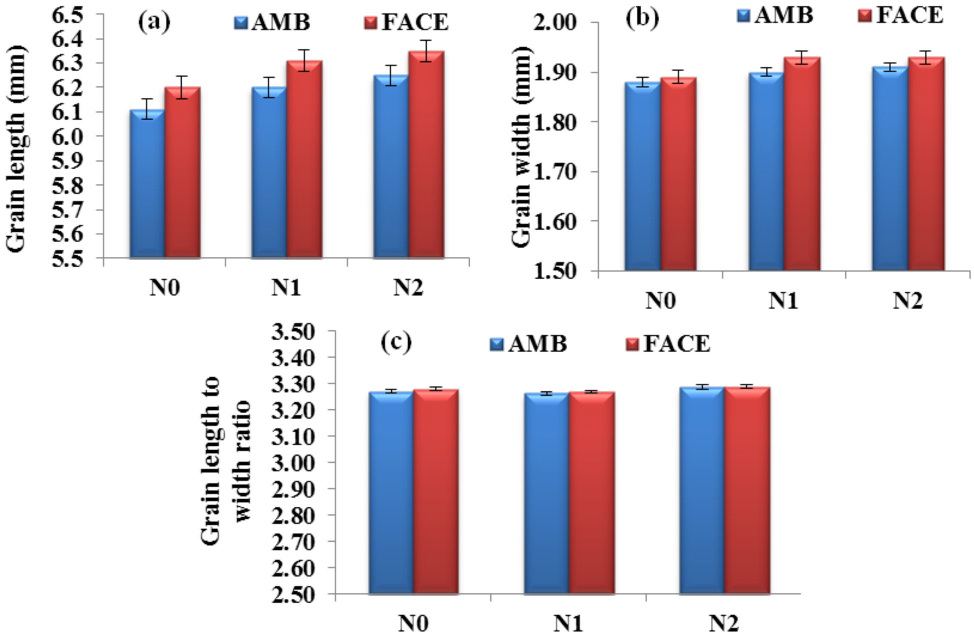

Figure 3 Impact of elevated CO_2 and N levels on appearance quality parameters of milled rice - (a) Grain length, (b) Grain width and (c) Grain length to width ratio

The AC was represented as the percentage of amylose to the milled rice biomass. When averaged over the N levels result showed that there was a significant increase of AC in milled rice by 14.2% under FACE facility. An experiment resulted in a 12.5% increase in amylose content in high CO_2 grown rice compared to ambient condition (**Figure 4a)** [27]. Although there were no significant impact of N doses on AC but it increases with increasing N doses. Generally, it is found that higher the AC contents relative to amylopectin higher will be the hardness of the cooked grains [23]. So as per our result elevated CO_2 condition is likely to enhance the hardiness of cooked grain. Similar result has been reported by some scientists [11] but contrast was there with others [9]. The reason behind the inconsistency in the results probably resides in use of different test varieties and/or prevailing environmental conditions. Yang [9] used a cultivar with AC of ca. 150 mg/g, Seneweera [11] used a high-AC variety with AC of ca. 300 mg/g or higher whereas we selected an intermediate-AC rice variety with AC of ca. 220 mg/g.

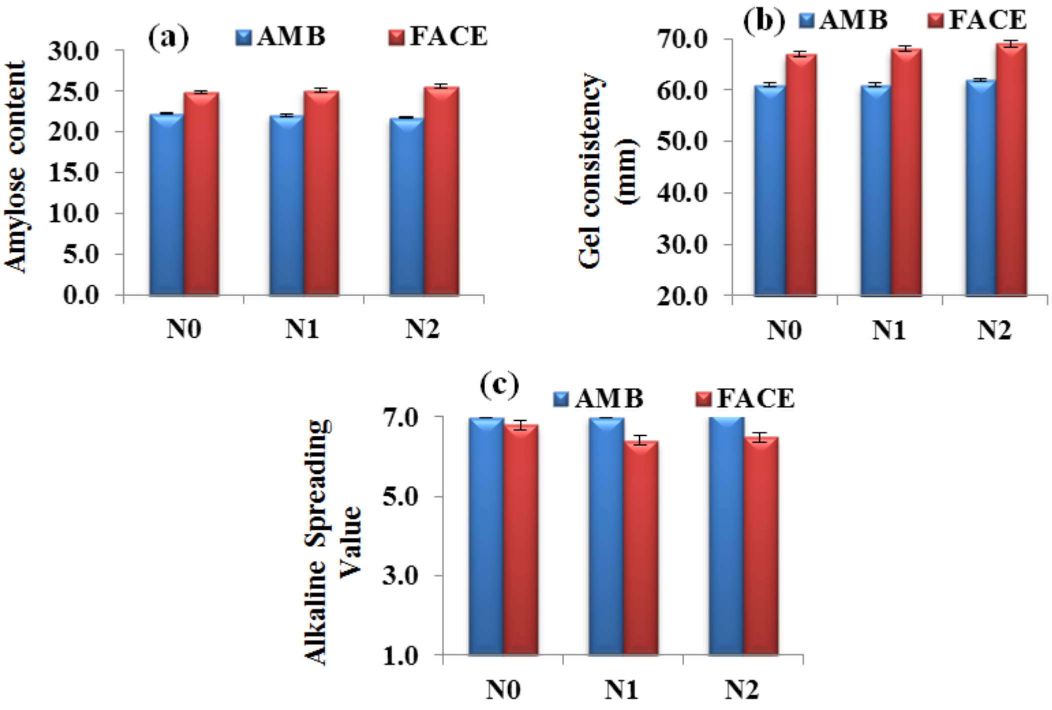

Figure 4 Impact of elevated CO2 and N levels on cooking quality parameters of milled rice - (a) amylose content, (b) gel consistency and (c) Alkaline Spreading value for estimating gelatinization temperature

GC corresponds to the length of cold milled rice paste in a test tube placed in a horizontal position. A higher value points to a softer consistency. GC showed a significant increase (10-11%, $P > 0.05$) (Figure 4b) in FACE grains compared to the ambient condition. Across CO_2 levels, varying the supply of N significantly influenced GC with greatest GC occurring at N2.

GT represents the temperature at which about 90% of the starch granules have swelled irreversibly in hot water. GT value can also be determined by Alkaline Spreading Value (ASV) which is inversely proportional to GT. Low ASV correspond to high GT i.e. high temperature is required for cooking. In present study ASV of Pusa 44 decreases in FACE condition as compared to ambient CO_2 level irrespective of N levels. The mean ASV was decreased by 6.6 in FACE compare to 7 in ambient condition. Further highest decrease in ASV was found with N1 dose from 7 to 6.4 (Figure 4c). The decreased ASV shows that grain produced under high CO_2 condition will produce harder grains and will require high cooking temperature future climate change scenario with high CO_2 concentration.

Nutritional quality

Rice being one of the most staple food crops of the Asian continent is a major source of dietary protein. So the nutritional property of the rice crop should be considered for the impact of elevated CO_2 under the present climate change scenario.

In the present study, concentration of mineral nutrients such as nitrogen (N), phosphorus (P), potassium (K), iron (Fe), and zinc (Zn) were also quantified in the milled rice fertilized with varying N doses both under ambient and elevated CO_2 conditions (**Table 2**). The results showed that the nitrogen content of milled rice enhanced gradually with increase in N levels. However, the concentration of P and K in milled rice gets reduced markedly with increased level of N application. Contrary to these, the concentration of micronutrients such as Fe and Zn in milled rice enhanced marginally with increased N doses. The concentration of both macro (N, P and K) and micronutrients (Fe and Zn) in milled rice grain however, was invariably lower under FACE facility as compared to the ambient condition. Reports indicate that most of C3 plants are able to capitalize on elevated CO_2 condition and convert it into photosynthetic rates and, consequently improved their growth and yield [4, 28].

Regardless of different N levels, protein content in milled rice was found to be reduced significantly under elevated CO_2 condition. Averaged over N levels, FACE reduced the protein content by about 10%. On the other hand, protein yield exhibited an opposite trend showing a significant increase of 11% under FACE (Table 2) which was due to the significant increase in grain yield due to CO_2 enrichment [9]. Across [CO_2] levels, both Protein content and protein yield increased significantly with increase in N supply and maximum value attained at N2 level (Table 2). There was no significant interactive impact of the two factors.

Table 2 Impact of elevated CO_2 and N doses on nutritional quality parameter of milled rice

N Treatments	**CO_2 Treatments**	**N (%)**	**P (%)**	**K (%)**	**Fe (ppm)**	**Zn (ppm)**	**Protein (%)**	**Protein yield (g pot^{-1})**
N_0	AMB	1.02	0.28	0.68	20.20	23.30	6.40	2.66
	FACE	0.94	0.24	0.62	18.50	21.30	5.88	2.86
	% Change	-7.84	-14.29	-8.82	-8.42	-8.58	-8.13	7.52
N_1	AMB	1.51	0.26	0.65	23.10	23.90	9.46	4.90
	FACE	1.36	0.21	0.61	21.30	22.70	8.52	5.48
	% Change	-9.93	-19.23	-6.15	-7.79	-5.02	-9.94	11.84
N_2	AMB	1.56	0.25	0.60	23.50	24.30	9.75	5.28
	FACE	1.39	0.20	0.56	22.10	23.40	8.70	5.94
	% Change	-10.90	-20.00	-6.67	-5.96	-3.70	-10.77	12.50
ANOVA ($P= 0.05$)	N	**	**	**	**	**	**	**
	CO2	**	**	**	**	**	**	**
	N x CO2	NS	NS	NS	NS	NS	NS	NS

Probability levels are indicated by NS, * and ** for 'not significant', 0.05, and 0.01, respectively. (AMB - 550±20 ppm, EC and FACE - 390±20 ppm, AC)

In a meta-analysis report showed a 10% decrease in average protein concentrations of milled rice when CO_2 concentration increased from 315–400 to 540-958 ppm [29]. Many others have also reported similar decrease in protein concentration in milled rice under CO_2 enriched facilities [29-32], pointing towards a possible deterioration of basic grain quality parameter for end-use purposes. The mechanism supporting decreases in concentration of N and protein under elevated CO_2 condition is difficult to differentiate as growth at elevated CO_2 condition can affect multiple processes involved in N uptake and metabolism [33, 34]. But one mechanism that has often been used to support the result is its dilution effect by increase in concentrations of nonstructural carbohydrates leading to decreased N and protein concentrations which was also reported in other experiment [35]. Also N limitation leads to an increase of non-structural carbohydrates like starch and sugars [36].

Conclusion

From an agricultural viewpoint and with the change in climate scenario, atmospheric CO_2 concentration enrichment will have several consequences. Rising atmospheric CO_2 concentration is likely to impact grain quality of various crops. Thus it is vital to study its impact on the crop yield as well as related quality parameters. Overall, from the results presented here we concluded that processing quality, appearance quality and nutritional quality tended to decrease in rice grains exposed to elevated CO_2 condition. Amylose content and gelatinization temperature found to be reduced under enhanced CO_2 condition impacting directly to the reduced cooking quality. In order to maintain desirable quality characteristics in rice crop under the increasing atmospheric CO_2 concentration, the current recommended rates of N fertilization should be modified little bit which may help to cope up with the future climate change scenario.

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