Enhance germination, seedling growth and vigor of rice by increasing concentration of CO2

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Abstract: Rice is a staple food for more than 50% of the world's population. Asia produces and consumes about 90% of all. Carbon dioxide levels are rapidly increasing in the atmosphere from 270 ppm to 384 ppm in 2009 and then 401 ppm in 2016. Different studies show that increasing CO2 has a positive impact on rice by enhancing the photosynthetic rate while others claim that long them Co2 can mediate the adverse effect on rice. Different research has been done on rice under elevated CO2. Most of the studies concerned physiology and yield. As the effect of elevated CO2 on seedlings has not been studied yet, here we applied 800ppm CO2 for 8 days and collected data. The results showed that elevated CO2 significantly increased rice seedling growth, germination rate, vigor, and speed of germination which increased from 83% to 92%.

Keywords: Chlorophyll, photosynthesis, emergence energy, rice varieties, elevated CO2

Introduction

Rice is a staple food for at least more than half of the world's population. Peoples in East and Soth Asia, West Indies, the Middle East, Africa, and Latin America consume rice as the main staple food (FAO, 2017). With the increasing population in these regions (Coats, 2003; Bloom, 2011) rice demand is projected to grow up to 2.000 million metric tons by 2030 (FAO 2017).

The atmospheric concentration of CO2 is continuously rising. It had increased from 270ppm before the industrial revolution to 384ppm in 2013 respectively (Goufo et al., 2014; Leaky et.al., 2009). Predictions are that CO2 concentration will reach 550ppm by mid of 21st century and 800ppm by the end of 2100(Feng et.al., 2014; Long and Ort, 2010). Rice is one of the most important crops for human nutrition and taking advantage of elevated CO2 for increasing yield is necessary to ensure food security. Studies on rice have indicated that elevated CO2 increases photosynthesis, tiller number, biomass, and grain yield as well as plant nitrogen (N) uptake (Kim et.al., 2001). Other studies have shown, however, that the rise in temperatures and levels of CO2 may have serious adverse effects either directly or indirectly on the growth, development, and yield of rice (Lobell et.al., 2011). Which of these is critical for the early development of rice?

During germination, the plant has increased susceptibility to disease, injury, and environmental stresses such as heat, water logging, and elevated CO2 (Doni et.al., 2014a; Doni et al., 2014b). Reducing crop growth and yielding poor germination and consequent uneven seedling growth can lead to financial losses (Ghiyasi et.al., 2008). Furthermore, in many plant species, especially annuals plants, in which the life cycle is mediated via sexual reproduction, seed germination, and seedling establishment, the germination and speed of germination, have been reported to have a marked influence on the population and community dynamics (Menges, 1991; Baskin and Baskin, 1998; Clauss and Venable, 2000; Radford and Cousens, 2000; Edwards et.al., 2001a). Despite the central role of CO2 on inter-generational dynamics in annual plants had been reported in much literature, however, little is known about responses of reproductive success and seed germination of rice plants to CO2 enrichment, especially during the germination stage (Steinger et.al., 2000; Andalo et.al., 1996; Mohan et.al., 204). Therefore, the objectives of this study were to assess the role of CO2 in influencing rice seedling growth, germination, and vigor.

Materials and Methods

Seed materials

Rice seeds (*Oryza sativa* L.) of varieties MRQ74 and MR269 were obtained from Lintang Organic Farm (SRI Lovely), Sik, Kedah, Malaysia. Empty and undeveloped seeds are discarded by floating in tap water before the germination test. Seeds of both varieties were thoroughly washed with tap water to remove dirt and dust for 5 min. To avoid possible inhibition caused by toxins from bacteria or fungi, seeds were surface sterilized with 10:1 distilled water/ethanol (75% ethanol) for 5 min and then washed 6-7 times with tap water. Seeds of MRQ74 and MR269 were soaked in distilled water for 24 hours (Doni et.al., 2016).

Plant growth and maintenance

Rice seeds were grown in soil. Two rice varieties MRQ269 and MRQ74 (*Oryza sativa* L.) were tested. Two hundred seeds were sown in a plastic tray at a depth of 2-2.5cm and thinned to one plant per pot at the two-leaf stage. Seedlings were maintained watered conditions by spraying twice a day with foliar feed (Miracle-Gro, the Scotts Company, UK Ltd.) for 8 days after transplanting.

Experimental Design

Experiments were conducted in greenhouses with two different CO2 concentrations namely: elevated (800ppm) and ambient. All treatments consisted of 20 biological replicates. The elevated CO2 concentration was maintained at 800ppm in a glass house in a lab. The seeds grown in a normal glass house at ambient CO2 were used as a control.

Determination of growth parameters

Emergence energy, final germination percentage, shoot and root length of seedling, vigor index, and biomass were determined as described below:

Emergence energy (EE)

Emergence energy is the percentage of germinated seeds 3 days after germination started and the total number of seeds tested per treatment. It was computed 72 hours after the onset of germination by using the following expression (Basra et.al., 2005).

$$\text{Emergence energy (EE)}= \frac{Number\ of\ seedlings\ emerged\ after\ 72\ hrs.}{a\ total\ number\ of\ seeds\ sown} \times 100$$

Germination rate (GR)

The germination rate was calculated according to the equation described by (Ellis et.al., 1980).

$$\text{Germination }\%= \frac{total\ number\ of\ seeds\ germinated}{total\ number\ of\ seeds\ taken\ for\ germination} \times 100$$

Seedling length and biomass

Seeds were considered germinated with the appearance of both plumule and radicle. The length of plumule and radical measured in values of cm. The fresh weight was recorded on day 9 of germination. Then, the seedlings were oven dried at 70R°C for 48hrs. and the dry weight recorded to mg. All the data was subjected to differences among treatment means at the 5% probably level estimated according to Duncan Multiple Range Test (DMRT) (Steel et.al., 1997).

Seedling Vigor Index

The vigor of the seedlings was estimated according to the formula of Abdul-Baki and Anderson (1973). (Abdul naki and Anderson, 1973)

SVI= total seedling length (mm)×germination %

Estimation of chlorophyll

Chlorophyll (chlorophyll a, b, and total chl.) was determined by the method of Arnon (Arnon, 1949). Fresh leaves were cut off on the 8th day of germination and chopped into small pieces using scissors extracted with 20 ml of 80% acetone in a Falcon tube and stored in the dark for 48 hrs. Chlorophyll content was estimated spectrophotometrically at 645 and 663 mm.

Results and Discussion

CO2 positively affected germination and vigor in both of the rice varieties. The results showed that as the CO2 level increases, several changes occur in all the observations of rice seed germination and seedling growth. The highest mean values were recorded in all parameters in treatment (elevated CO2 800ppm) while the lowest mean values were recorded in control (ambient CO2). Maximum germination % (92%) was found for MR269 under elevated CO2 while low germination percentage (71.5%) was observed for MRQ74 at control plants.

Caemmerer et.al., 2012 reported a significant increase in canopy biomass, root biomass, grain yield, leaf area index, and net C assimilation rates of plants growing under elevated CO2 conditions. Similar results were also noted here. Seedling length for both varieties

Table 1. effects of elevated CO2 on rice seedling growth, vigor and germination (Var. MR269)

Measurements	Treatment	Control
Shoot length (cm)	22.92	18.25
Root length (cm)	37.50	35.50
Shoot weight(g)	72.00	59.00
Root weight (g)	38.00	35.00
Dry weight shoot (g)	11.85	8.65
Dry weight root(g)	14.35	11.30
Chlorophyll a (ppm)	3992.10	3564.00
Chlorophyll b (ppm)	1322.10	965.50
Total Chlorophyll (ppm)	5314.40	4511.30

Note: Mean followed by different letter of the same days of treatment is significant tested using DMRT at $p<0.05$

Was more expressed and gradually increased in root length with increased CO2 level (Table 1 and 2). Furthermore, De Costa et.al., (2003) found that elevated CO2 have slightly increased the leaf chlorophyll in rice. These results are in agreement with our present study that reported an increased in chlorophyll concentration in rice plants grown under elevated CO2 (Table 1and 2).

Table 2. Effects of Elevated CO2 on rice seedling growth, vigor and germination (Var.MRQ74)

Measurements	Treatment	Control
Shoot length (cm)	15.45	12.60
Root length (cm)	41.25	28.75
Shoot weight (g)	57.50	31.50
Root weight (g)	35.00	29.50
Dry weight shoot (g)	6.801	3.35
Dry weight root (g)	1.90	10.25
Chlorophyll a (ppm)	3410.40	2750.50
Chlorophyll b (ppm)	886.3	947.2
Total chlorophyll (ppm)	4296.8	3716.20

Note: Mean ± standard error (SE) followed by different letter of the same days of treatment is significant tested using Duncan multiple range test at $p<0.05$

Germination is a comprehensive physiological process affected by a number of associated processes, i.e., osmotic potentials, metabolic activities, water uptake, environmental stress, cell membrane regulation, and genetic information of the seed (Doni et.al.,2016). Higher final germination percentage, energy emergence rate, and germination speed of rice varieties were observed under elevated CO2 (Table 3). Seedling height, vigor index, and seedling weights (fresh and dry) are associated with plant health, water availability, root osmotic potential, environmental stress, metabolic activities, and CO2 availability (Islam and Karim, 2010). Turhan et.al., (2008) found that the reduction in fresh and dry weight of seedlings was due to poor CO2 fixation efficiency. In agreement with his study, we find out in our research that the fresh and dry weight of rice seedlings has been increased by increasing the concentration of CO_2. The highest seedling fresh and dry weight resulted for both the varieties under elevated CO_2 as compared to ambient CO_2 concentration (Table 1 and 2).

Table 3. Germination%, Emergence energy and vigor index of rice seedlings to elevated CO_2 treatment

Measurements	Var. MR269		Var. MRQ74	
	Treatment	Control	Treatment	Control
Germination%	92%	83%	71.5%	78%
Emergence energy	59	56	21	18
Vigor index	5374.64	4461.25	2935.85	4422.6

Rice has been considered one of the most sensitive crops. However, there are huge genetic differences in rice as it has been cultivated centuries ago in different geographic regions by a number of civilizations as a staple food. In previous century, many standard institutes and well known research centers have been developed for rice research and their contribution to the varietal development for acquired characters have diversified the rice genome (Anbumalarmathi and Mehta, 2013). For all germination and vigor parameters studied in this research, highly significant variations among rice varieties under elevated CO_2 were revealed.

Conclusion

The result of this experiment indicates that high CO_2 has positive impact on rice seed germination, vigor index and early seedling growth; however different varieties (MR269 vs. MRQ74) were noticed to respond differentially towards elevated CO_2. All parameters observed were highest in MR269 variety under elevated CO_2, followed by MRQ74 variety under elevated CO_2 and control plants (MR269 and MRQ74 varieties grown under ambient CO_2).

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