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RESEARCH ARTICLE

IMPACTS OF RECENT AND ANTICIPATED CHANGES IN ATMOSPHERIC CO_2 CONCENTRATIONS ON GROWTH PERFORMANCE OF WEEDY RICE (*ORYZA SATIVA* F. *SPONTANEA*) BIOTYPES IN SRI LANKA

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ABSTRACT

Weedy Rice (WR) competes with cultivated rice and leads to loss of yield. The differential growth responses of WR and cultivated rice to increasing CO_2 are important in controlling and managing WR. The effect of changing atmospheric CO_2 concentrations on growth responses of WR-biotypes and cultivated rice (CR) using three WR biotypes (Wet, Intermediate and Dry climatic zones) and cultivated rice variety Bg300 in a growth chamber with varying concentrations of CO_2 (300, 400 and 500 µmol mol^{-1} 24 hd^{-1}) was tested. Data were collected at vegetative stage at 20, 30 and 54 Days after sowing (DAS). Destructive sampling was used for biomass determination. Differential sensitivities of WR-biotypes and CR-Bg300 to increasing CO_2 concentration was observed for leaf-width, leaf-area, number of culms and number of leaves, root biomass and total biomass. Elevating CO_2 significantly increased vegetative growth and biomass-accumulation in WR biotypes than Bg300 showing higher physiological plasticity in WR biotypes contributing to the wide occurrence of WR in rice fields in Sri Lanka.

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INTRODUCTION

Plants were the first group of organisms possessing mechanisms enabling them to synthesize complex carbohydrates and store chemical energy that cropped from the sources of sunlight, water and CO_2 and had been progressed through a course of evolution for 2.5 - 3.5 billions of years before (Farquhar *et al.*, 2010). Early plants thrived in the higher atmospheric CO_2 concentrations 3 to 4 times higher than the present values and in lower concentrations for a period of past 25-30 million years (Franks *et al.*, 2013). Recently, a considerable increase in the atmospheric CO_2 concentrations has been observed and an increase of CO_2 is 21% (from 315 to 382 µmol mol^{-1}) since 1960 (Keeling and Whorf, 2005) and is anticipated to be increased in the range of 550-850 µmol mol^{-1} by 2100 (Franks *et al.*, 2013) (Franks *et al.*, 2013). The CO_2 concentration was in the steady state at 280 µmol mol^{-1} during the pre-industrial period (before 1850) and on an annual basis there is an increase in atmospheric CO_2 concentration at the rate of 1.5 to 1.8 µmol mol^{-1} per year. The changes in atmospheric CO_2 exert profound impacts on both climate and functions of plants.

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Background/ Literature Survey

Studies are being conducted to understand the nature of feedbacks of plants at different scales within different vegetation systems in an effort to predict the general behavior of plants under changing CO_2 (Norby *et al.*, 1999; Amthor, 2001; Ainsworth and Rogers, 2007; Gerhart and Ward, 2010). More importantly, increase of CO_2 brings additional impacts on agricultural productivity which is already constrained under increasing pressure of global anthropogenic changes, including rising population, diversion of cereals to biofuels, increased protein demands and climatic extremes. Owing to the immediate and dynamic nature of these changes, adaptation measures are urgently needed to ensure both the stability and sustainability of the global food security (Ziska *et al.*, 2012). Rice (*Oryza sativa* L.) is an important source of carbohydrate for the human population in the tropical countries. Differential responses of the rice crop and associated weeds to rising CO_2 may have significant implications with respect to rice production and subsequent food security. Differential feedbacks of crops and weeds are evident under the changing atmospheric CO_2 concentrations (Hatfield *et al.*, 2011; Ziska *et al.*, 2014). Rice cultivating areas in several countries in South East Asia are seriously affected by the presence of several weeds. The most troublesome weeds in rice fields belong to Poaceous genus *Echinochloa* and are of C_4 category. Recently, there is growing concern on a new weed, weedy rice (*Oryza sativa* f. *spontanea*) which is spreading rapidly in the rice growing areas of the world. WR reduces crop yields and affects quality of yield to a greater extent than *E. crus-galli*. Yield loss due to the completion of WR ranged from 16-74% (Azmi *et al*., 2003) and even be as much as 80% (Smith, 1988). Presently, WR has already been a major problem and prevails as a noxious weed throughout the world, more prominently in direct-seeded rice cultivation. WR was first reported in the Eastern Province of Sri Lanka in early 1990s and is at present spreading into many rice growing areas irrespective of the agro-ecological zones of the country (Abeysekera, 2010). As far as Sri Lanka is concerned, diversity of WR has been increased from a few to more than 1300 different biotypes during the past two decades (Abeysekera *et al*., 2010). WR is currently considered as one of the most troublesome, difficult-to-manage and economically damaging weed problems in Sri Lanka (Marambe, 2009). WR belongs to the same species of cultivated rice and share the same genome (AA). The origin of the weedy forms of rice is closely associated with the modern cultivars of rice (*O. sativa*) (Song *et al*., 2014). WR shares a number of characteristics with other successful weeds meanwhile possessing unique phenological and morphological characteristics of cultivated rice. Due to these similarities, there were no selective herbicides available to control WR in rice paddies.

Due to the limited number of reports available on impact of increased atmospheric CO_2 concentrations studies on growth performance of WR and CR are essential to have a clear picture of the growth performance of WR and CR under varying atmospheric CO_2 concentrations. Thus, it is worth to investigate the responses of cultivated rice varieties and local WR biotypes to changing CO_2 concentrations *via* growth parameters such as vegetative and reproductive characteristics, and gene transfer potentials among close relatives to past, present and projected atmospheric CO_2 concentrations. The present study focuses on the responsive growth performance of three WR biotypes collected from three different climatic zones and widely cultivated inbred-rice variety, Bg300 in Sri Lanka to changes in atmospheric CO_2 concentrations. Thus, it was hypothesized that there were differential response to the increasing atmospheric CO_2 concentrations in the context of improving early competitive ability of weeds relative to cultivated rice.

MATERIALS AND METHODS

Study sites and Materials

Three WR biotypes (MW-Matara, KW-Kurunegala and AW-Anuradhapura) collected from three different climatic zones (Matara (wet zone), Kurunegala (intermediate zone), and Anuradhapura (dry zone) Districts) in Sri Lanka (Table 1), and one inbred-cultivated rice variety, Bg300 (as a reference) have been used for the study.

Methods

A controlled environment chamber was used because, at present, there is no methodology available to expose plants to sub-ambient CO_2 for 24 h d^{-1} (Mayeux *et al*., 1993). Temperature was varied in a diurnal mode from an overnight low of 22 °C, to a maximum afternoon value of 32°C, with an average daily (24 h) value of 24.5 °C. Light as photo synthetically active radiation (PAR) varied diurnally in conjunction with temperature, with the highest PAR (~900 µmol m^2 s^{-1}) occurring during the afternoon. The daily light period was 14 h, supplied by a mixture of high-pressure sodium and metal halide lamps, and averaged 22.3 mol m^{-2} d^{-1} for the chamber.

Growth Chamber Experiment

The concentrations of injected CO_2 or CO_2–free air in the chamber were determined taking gas samples and analyzing them in a GC analyzer (GC-8A, Shimadzu, Kyoto, Japan). The CO_2 concentrations were set at 300, 400 and 500 µmol mol^{-1} 24 h d^{-1}. These concentrations approximated the CO_2 values present during the middle of the 20th century, present as well as that projected to occur in the next 30 to 50 years (Keeling and Whorf, 2005). Temperature was kept at constant and humidity was recorded every 1hr and averages were determined on a daily basis for all experimental runs. Light intensity kept at ambient conditions. Seeds of three different WR biotypes collected from three climatic zones in Kurunegala, Matara and Anuradhapura Districts of Sri Lanka and rice variety, Bg300 (as a reference) were used for the experiment conducted under controlled environmental chambers. Ten seeds of each WR biotype and Bg300 placed in 1.0L pots in four replicates and thinned to four seedlings, 4-6 days after emergence. Ten seeds of each WR biotype were sown in 1.0 L pots filled with soil collected from the respective sampling sites and thinned to four seedlings 4-6 days after emergence for each CO_2 treatment. The data were collected at each of the two phenological stages (i.e. vegetative and reproductive). There was one non-destructive sample (NDS) (one replicate from each 4 Block) and 3 destructive (DS) samples (1 replicate from each 4 Block). The destructive plant samples were selected at random from each 4 Block. Before harvesting started (destructive samples) one plant was selected randomly from each block and tagged as the non-destructive sample. A randomized complete block design (RCBD) was used with four replicates (4 Blocks, four replicates in each block four replicates). Since the experiment was not continued up to physiological maturity of the plants, differences in initial growth, specially the leaf development, which was reported to be a good predictor of competitive outcomes between weeds and crops, were considered in the present study (Keeling and Whorf, 2005). The first, second and the third sampling were destructive and at each destructive sampling, the parameters; Seedling Height, Leaf Blade Length, Leaf Blade Width, Number of Leaves, Culm Number, Shoot biomass and Root biomass were measured. Leaf area was calculated according to the length-breadth method (Palaniswamy and Gomez, 1973). The dry weights were determined separately by oven-drying to constant weight at 80°C. The plant biomass was computed by summing up the dry weight of different plant parts and expressed as g/plant.

Supply of CO_2 to Growth Chamber

Pure CO_2 (98%) was injected to the Growth chamber through a Teflon tube connected through a regulator to a cylinder. The flow rate of CO_2 (300, 400 and 500 µmol mol^{-1}) was controlled manually through a valve to maintain the CO_2 concentration within the Growth Chamber. Temperature, humidity and light intensity in the Growth Chamber were adjusted to ambient conditions. Gas samples were collected from the chambers to ascertain the CO_2 concentration within the chamber and analyzed using Gas Chromatography.

Statistical Analyses

All measured and derived parameters were analyzed using ANOVA and GLM procedures on SPSS PC, Ver. 20 (2010). The CO_2 concentrations and WR biotypes and the reference rice variety (Bg300) were taken as factors and growth parameters as dependent variables.

RESULTS AND DISCUSSION

The responses of the initial growth and leaf area (i.e., from seedling upto flag leaf development) of the WR biotypes and CR-Bg300 under different CO_2 concnetrations were shown in Figure 1. There was no discernible difference among the WR biotypes and CR-Bg300 at 300 and 400 µmol mol^{-1} upto 40 DAS (DAS- Days after sowing). However, apparaent differences were observed at 54 DAS under both CO_2

Table 1. Districts and locations (GPS position) of weedy rice (*Oryza sativa* f. *spontanea*) biotypes collected in Sri Lanka

District	Location	Weedy rice biotype
Kurunagala	KurunegalaBulunAhala Yaya (7^{0}38' 48.3" N80^0 30' 28.8" E)	KW1
	KumbukwawaDahampalaYaya (7^{0}35' 19.2" N80^0 28' 38.4"E)	KW2
	KuliyapitiyaHambalawaYaya (7^0 27' 20.1" N 80^0 5' 15.8" E)	KW3
Matara	MataraWeligamaMudugamuwa (6^0 06'66.5"N 80^0 57' 75.5" E)	MW1
	MataraMapalanaKamburupitiya (6^0 02' 74.4" N 80^{0}57' 50.9" E)	MW2
	MataraPalolpitiyaAkurugoda (6^0 84' 62.2" N 80^0 65' 2" E)	MW3
Anuradhapura	Anuradhapura Kunchikulama (08°21'52.3N,80°33'32.5E)	AW1
	Anuradhapura Puliyankulama (08°20'43.2N,80°23'14.3E)	AW2
	Anuradhapura Shrawasthipura (08°22'12.5N,80°21'22.5E)	AW3

Table 2. Selected vegetative characters (per plant) for weedy rice biotypes (AW, KW, MW) and cultivated rice Bg300 variety and their relationship between varying atmospheric CO_2 concentrations (300, 400, or 500 µmolmol^{-1}) at panicle initiation stage (ca. 50 DAS). Averages for variables with statistically significant interactions between CO_2 and rice variety are also indicated

	Averages			Rice variety				P values		
Variable	300 µmol mol^{-1}	400 µmol mol^{-1}	500 µmol mol^{-1}	AW	CR	KW	MW	CO_2	PT	CO_2 * PT
Shoot biomass (g/plant)	8.05	12.36	13.06	14.13	9.27	10.14	11.08	***	***	***
Plant height (cm)	47.48	47.66	48.54	49.85	47.62	45.84	48.26	ns	***	ns
Leaf blade length (cm)	33.88	34.29	35.55	35.63	36.89	32.27	33.5	*	***	ns
Leaf blade width (mm)	4.53	5.44	6.07	5.57	5.15	5.57	5.09	***	***	***
Number of leaves / plant	4.96	5.43	6.32	5.39	5.48	5.56	5.85	***	***	*
Number of culms per plant	2.54	2.56	4.29	3.17	2.94	3.11	3.3	***	***	***
Shoot weight per tiller (g)	2.59	2.66	1.55	2.52	1.53	2.45	2.56	***	***	ns
Leaf area (cm^2 / plant)	4288	5692	7645	6130	5861	5738	5773	***	***	***
Total biomass (g/plant)	11.23	15.7	16.55	17.01	11.9	13.53	15.52	***	***	***
Root biomass	3.17	3.34	3.49	2.89	2.63	3.39	4.43	ns	***	ns
Estimated mean values for variables with significant interactions between $CO_2$and plant type										
Shoot biomass (g /plant)										
AW	10.577	14.367	17.433							
CR	6.05	11.233	10.527							
KW	7.25	14.467	8.717							
MW	8.333	9.367	15.553							
Leaf bade width (mm)										
AW	4.61	5.56	6.56							
CR	4.94	5.5	5							
KW	4.5	5.61	6.61							
MW	4.06	5.11	6.11							
Number of leaves per plant										
AW	4.83	5.17	6.17							
CR	5	5.44	6							
KW	5	5.33	6.33							
MW	5	5.78	6.78							
Number of culm per plant										
AW	2.5	2.5	4.5							
CR	2.61	2.61	3.61							
KW	2.44	2.44	4.44							
MW	2.61	2.67	4.61							
Total leaf area (cm^2)										
AW	3779.46	5339.91	9294.56							
CR	4520.83	6022.67	6753.00							
KW	3532.50	5122.13	8894.66							
MW	3325.42	5794.48	9798.81							
Plant biomass (g per plant)										
AW	17.24	13.28	20.52							
CR	8.56	13.89	13.26							
KW	10.46	17.86	12.27							
MW	12.61	13.82	20.13							

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
ns = not significant at $p > 0.05$

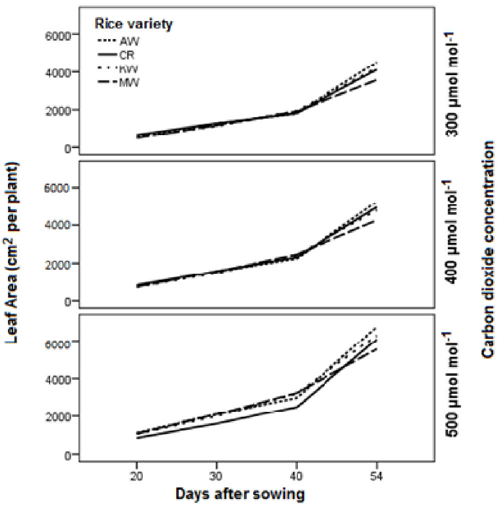

Figure 1. Variation of leaf area per plant of weedy rice biotypes (AW, KW and MW) and cultivated rice (Bg300) grown at three levels of CO_2 concentrations (300, 400, and 500 μmolmol^{-1})

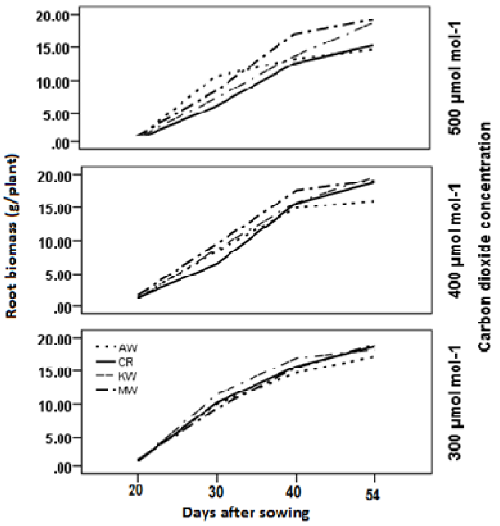

Figure 2. Variation of root biomass (g / plant) of weedy rice biotypes (AW, KW, MW) and cultivated rice (Bg300) in response to varying CO_2 concentrations (300, 400, and 500 μmolmol^{-1})

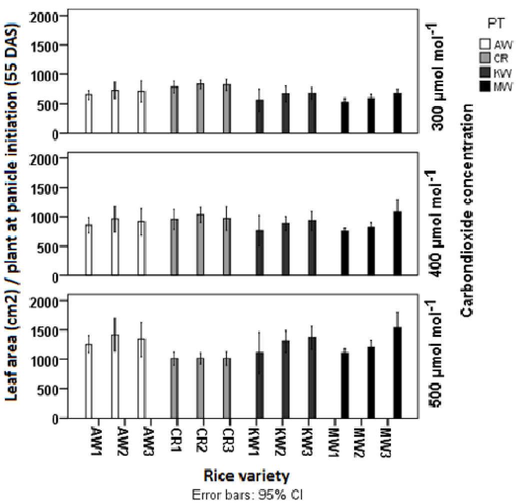

Figure 3. Variation of leaf area at panicle initiation, ca. 55 d after sowing (DAS), for individual weedy rice biotypes (WR biotype) and Bg300 variety at three different levels of CO_2 concentrations (300, 400, and 500 μmolmol^{-1}). For average values for WR biotypes and Bg300 are given for each CO_2 concentrations. Significant CO_2 concentration × type interactions were observed as a function of increasing atmospheric CO_2 (refer Table 2)

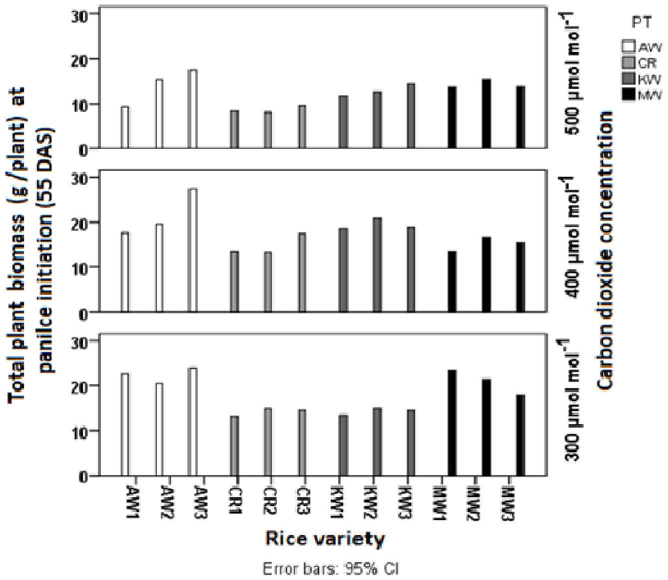

Figure 4. Summary of the variation of total biomass at panicle initiation, ca 55 days after sowing (DAS), for individual WR biotypes and Bg300 variety at three different levels of CO_2 concentrations (300, 400, and 500 μmolmol^{-1}). For average values of WR biotypes and CR varieties are given for each CO_2 concentration. Significant CO_2 concentration × type interactions were observed as a function of increasing atmospheric CO_2 (refer Table 2). Similar to the leaf area, significant interactions between CO_2 concentration and total biomass were observed between WR and CR as a function of rising CO_2 level

concnetrations (300 and 400 μmol mol^{-1}). WR biotypes AW and KW indicated a higher values of leaf area than CR-Bg300 and lowest value of leaf area was observed for MW. The variation of leaf area at the highest level of CO_2 concnetration (500 μmol mol^{-1}), indicated a considerable difference between the WR biotypes over CR-Bg300 which was higher at 40 DAS. The root biomass reflects a similar trend which was observed for leaf area. However, differences appeared even at 30 DAS and was prominent at 54 DAS. Differentiation between WR biotypes and CR-Bg300 appeared to take place earlier with increasing CO_2 concnetrations. A significant difference between WR biotypes and CR-Bg300 was observed for leaf area at 500 μmol mol^{-1}, CO_2 concentration at 30 DAS. Significant differences in leaf area and root weight were observed at 30 DAS, whereas at 300 and 400 μmol mol^{-1} CO_2 concentrations, such significant difference was not observable until 40 DAS (Figure 1). A similar trend was indicated in the root biomass in which differential responses of WR biotypes and CR-Bg300 at *ca.* 30 DAS at 300 μmol mol^{-1} of CO_2 concentration (Figure 2). With increasing CO_2 concnetrations, a difference in root biomass between the WR biotypes and CR-g300 observed even at 25 DAS at 400 and 500 μmol mol^{-1} CO_2 concnetrations. The differences are much more prominnent at the 500 μmol mol^{-1} CO_2 concnetration and in WR biotypes the root biomass exceeded the root biomass of CR-Bg300.

Variability in measured growth parameters of WR biotypes and different levels of CO_2 were shown in Table 2. The panicle initiation (ca. 54 DAS), was differed significantly between WR biotypes and CR-Bg300 were observed for shoot biomass, leaf blade width, lead blade length, number of leaves per plant, number of culms per plant, shoot weight per tiller, leaf area and total biomass. In addition, interaction of rice variety × CO_2 concentrations were statistically significant for the variables such as shoot biomass, leaf blade width, number of leaves per plant, number of culms per plant, leaf area and total biomass (Table 2). Complying with the results observed for the initial growth, most of the WR biotypes had a greater response to rising CO_2 than CR-Bg300. Variability in the response of individual genotypes was also observed in CR-Bg300 and within WR biotypes with respect to two important growth parameters such as total plant biomass and leaf area at panicle initiation (Figures 3 and 4). Comparatively, a higher phenotypic difference was observed for WR biotypes compared to CR-Bg300 for leaf blade with, number of leaves per plant, tiller weight as well as leaf area (higher in CR-Bg300) suggesting greater variability for these parameters between WR biotypes and CR-Bg300 varieties. However, in contrary, among WR biotypes, a considerable variability, particularly in plant height, leaf blade length, number of culms per plant were observed. For instance, at 300 µmol mol^{-1}, average total plant biomass and leaf area for CR-Bg300 were 21% and 57% greater than WR biotypes. However, at 500 µmol mol^{-1} CO_2 concentration, the relative responses of WR biotypes over CR-Bg300 were 38% and 33% for the same vegetative parameters (Figures 3 and 4).

The results obtained for the present study limits to growth parameters, because study was not extended to the seed-setting stage of WR biotypes and CR-Bg300. At the initial stages, response of growth parameters such as leaf area to baseline CO_2 concentrations and even for 400 µmol mol^{-1} was weaker for both WR biotypes and CR-Bg300 variety. However, with the increase in CO_2 concentration (e.g. 500 µmol mol^{-1}), leaf area was increased even at the initial stages of the plant growth. Meanwhile, root biomass indicated a considerable increase in early root development (Figures 1 and 2). The increase in root biomass in early growth stage suggests that WR biotypes promptly react to the increasing CO_2 concentrations in the atmosphere favoring competitive advantages for WR biotypes over CR-Bg300. A significant interaction between the CO_2 concentration and rice variety were observed for a number of growth parameters such as biomass, leaf area at the panicle initiation (*ca.* 54 DAS) ($p \leq 0.05$). The significant interaction between plant type and CO_2 clearly indicated that WR biotypes benefited over CR-Bg300 with respect to increasing CO_2 concentration (Table 1). This ability of WR biotypes in responding to increasing CO_2 concentration imply that WR biotypes could impose a greater production limitation in CR-Bg300 with elevating atmospheric CO_2 concentration (Sales *et al*., 2011). The WR biotypes have shown that they possess higher capability over cultivated CR in responding to increasing CO_2 concentrations at 300 µmol mol^{-1} levels. Though, the origin of this capability is uncertain, it suggests a higher degree of genetic and phenotypic plasticity between WR biotypes and uniform, early in maturing, and short stature cultivated rice varieties. However, the responsible genes of these variations in WR biotypes could be used to breed cultivated rice varieties to utilize the increase in atmospheric CO_2. The findings of the present study are in agreement with Ziska *et al*. (2012) and Ziska *et al*., 2014).

Conclusions/Future work

The increasing anthropogenic activities which expedite the changes in atmospheric CO_2 concentrations, are potential in changing agricultural productivity in variety of ways. In the present work it was assessed effect of the recent and projected changes in atmospheric CO_2 as the source of carbon for plant growth, which could differently affect the growth and yield of weedy rice and cultivated rice varieties. The results showed that weedy rice ecotypes could demonstrate a stronger response to rising CO_2 than its cultivated rice, with higher competitive ability, and exerting subsequent negative effects on cultivated rice yields. Though the results are preliminary, they provide additional information on the impact of increased atmospheric CO_2 on weedy rice–cultivated rice competition is apparent. It is expected to repeat the same experiments considering the impact of recent and anticipated changes in atmospheric CO_2 as well as and temperature on growth performance on weedy rice.

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