INFLUENCE OF ELEVATED CO_2 ON ZINC DYNAMICS IN RICE (*Oryza sativa* L.)

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(*Oryza sativa* L.)

by

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KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **“Influence of elevated CO_2 on zinc dynamics in rice (*Oryza sativa* L.)”** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Certified that this thesis entitled **“Influence of elevated CO_2 on zinc dynamics in rice (*Oryza sativa* L.)”** is a record of research work done independently by Ms. Ramireddy Bhavana under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

Zn	Zinc
WHO	World Health Organization
Fe	Iron
UNFAO	United Nations Food and Agriculture Organization
Carbondioxide	CO_2
eCO_2	elevated CO_2
OTC	Open Top Chamber
AMF	Arbuscular Mycorrhizal Fungi
Mha	Million hectares
kg/ha	Kilogram per hectare
NOAA	National Oceanic and Atmospheric Administration
C_3	Calvin cycle
C_4	Hatch and Slack Pathway
Rubisco	Ribulose bisphosphate carboxylase/oxygenase enzyme
HI	Harvest Index
FACE	Free air CO_2 enrichment
TGC	Temperature gradient type CO_2 concentration control chamber
DAT	Days after transplanting
avg	Average
ppm	parts per million
$\mu mol\ mol^{-1}$	micromole per mole
$\mu mol\ CO_2\ L^{-1}$	micromole CO_2 per litre
$\mu LCO_2\ L^{-1}$	microlitre CO_2 per litre
Pa	Pascal
gm^{-2}	gram per square metre
$gpot^{-1}$	gram per pot
Pmax	Light saturated photosynthetic rate

µmol CO_2 $m^{-2}s^{-1}$	micromole CO_2 per square metre per second
mmol H_2O $m^{-2}s^{-1}$	millimole H_2O per square metre per second
gs	Stomatal conductance
SPAD	Soil Plant Analysis Development
g/plant	Gram per plant
%	per cent
^0C	Degree Celsius
cm	Centimeter
g	Gram
mg	Milligram
ml	Milliliter
µl	Microliter
mg g^{-1}	Milligram per gram
µg	microgram
µg g^{-1}	Microgram per gram
mM	millimolar
nm	Nanometer
DW	Dry Weight
m^{-2}	Square metre
v/v	volume per volume
w/w	weight per weight
et al.	and other co-workers
viz	namely
i.e.	that is
FYM	Farm Yard Manure
kg	kilo grams
$ZnSO_4$	Zinc sulphate
N	Nitrogen
K_2O	Potassium oxide
P_2O_5	Phosphorus pentoxide
IPCC	Intergovernmental Panel on Climate Change
UV	Ultra violet

CRD	Completely Randomized Design
KAU	Kerala Agricultural University
PP system	Portable Photosynthesis system
U.S.A	United States of America
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediamine tetraacetic acid
BSA	Bovine Serum Albumin
DNS	Dinitro salicyilic acid
ANOVA	Analysis of Variance
OPSTAT	Operational Statistics
CD	Critical Difference
SE(m)	Standard Error (Mean)
G0	Gap zero
G1	Gap one
DNA	Deoxyribo Nucleic Acid
RNA	Ribonucleic Acid
Os ZIP5	Rice Zn regulated transporter-like protein5

Introduction

1. INTRODUCTION

Zinc is an essential trace element that plays a very crucial role in many physiological functions in human beings, plants and animals. One of the essential functions of Zn is its influence on the immune system. It is required for the growth, development and functioning of immune cells in the innate and the adaptive immune system. Zn homeostasis is finely controlled within each cell and any deregulation results in impairment of normal functions and it is an excellent anti-inflammatory immunomodulator. Consequences of impaired homeostasis can be noticed in many disease models such as infections, allergies, autoimmune diseases, and cancers. The deficiency of Zn negatively influences the hematopoiesis and compromises the immune response at multiple molecular, cellular, and systemic levels (Gammoh and Rink, 2019). Zn deficiency also limits growth and development in infancy and childhood. Severe deficiency may be observed in preterm infants especially if necrotizing enterocolitis coexisted (Gamal and Mahmoud, 2020).

Appropriate Zn intake is the cornerstone of global maternal and child health and 17% of the world's population was supposed to be at a risk of deficiency of Zn in 2011 (Wessells *et al*., 2012). The total population estimated to be placed at new risk of Zn deficiency by 2050 is 138 million. The people who are most affected live in Africa and South Asia, of which nearly 48 million are residing in India alone. The global burden of disease attributed to Zn deficiency is high, with greater than 100,000 deaths per year in children younger than 5 years (Black *et al*., 2013). WHO (2017) reported that annual mortality of 4, 33,000 children under the age of five is due to the deficiency of Zn.

Cereals are the major source of Zn for the global population, particularly for the poor residing in rural areas. However, the Zn content of cereal-based foods is quite insufficient to meet human requirements (Boonchuay *et al*., 2013). C_3 grains and legumes come up with more than 60% of dietary Zn and/or Fe for a population of around 2 billion (Myers *et al*., 2014).

Among these crops, rice is an important staple food for half of the world's population which supplies 30% of total protein for 3,400 million individuals (UNFAO, 2011). It is the predominant dietary energy source for the people of East and South-East Asia and also for three-fourths of Indian population "rice is life" as it plays a crucial role in the country's food security (Babu *et al.,* 2015). India is having a rice growing area of around 42.95 Mha, having a production of 112.90 MT with the productivity of 2699 kg/ha (Directorate of Economics & Statistics, 2018).

Changes in the environmental variables such as high temperature and atmospheric carbon dioxide (CO_2) concentration directly or indirectly affect plant growth, development, grain yield, and quality of crops (Fernando *et al*., 2012; Thilakarathne *et al*., 2013; Panozzo *et al*., 2014). Since the industrial revolution, the rise in CO_2 has been reported and is expected to increase to a greater extent in the middle of the century (IPCC, 2014).

Global CO_2 emissions continue to rise year by year, reaching the highest concentrations of atmospheric CO_2 recorded 405 ppm in 2017. The recent increase in atmospheric CO_2 has reached to 414.11 ppm as on February 2020 (NOAA, 2020).

Quantitative results of different studies demonstrated that elevated CO_2 (eCO_2) stimulated the grain yields of many crops (Al-Hadeethi *et al*., 2019). However increasing concentration of CO_2 in the atmosphere is expected to bring down the content of essential elements like protein, Zn and Fe in cereals and other crops which may threaten the nutrition of billions of people in the upcoming years (Ujiie *et al*., 2019). In addition to dietary shifts away from micronutrient-rich coarse cereals in the Indian diet, anthropogenic CO_2 emissions are likely to worsen these trends in the future. In this scenario, attempts were made to evaluate the field performance of popular rice varieties of Kerala and also to enrich Zn status of grains under increasing CO_2 condition. Zn concentration of rice (*Oryza sativa* L.) grain is enhanced by foliar Zn application (0.5% $ZnSO_4$). The role of AMF (Arbuscular Mycorrhizal Fungi) in increasing water and nutrient uptake, especially phosphorus, zinc and copper, is well known (Bowen, 1973). They are also the key factors for successful low-input farming

(Johansson *et al*., 2004). Priming of seeds in solutions of macro or micronutrients enhances the yield of rice (Peeran and Natanasabapathy, 1980).

Based on these backgrounds, the present programme “Influence of elevated CO_2 on Zn dynamics in rice (*Oryza sativa* L.)” was formulated with the objective of assessing the impact of elevated CO_2 on growth, development and nutritional quality of rice in relation to modifications in Zn dynamics. Plants were raised under different CO_2 conditions along with Zn enrichment techniques. The information generated will help farmers to raise quality produce with the help of low cost technologies and to make paddy cultivation more profitable. The research outcome can also be an answer to the serious health crisis and malnutrition faced globally in this changing climatic scenario.

Review of Literature

2. REVIEW OF LITERATURE

In the present study an attempt has been made to study the impact of elevated CO_2 (eCO_2) on growth, development and nutritional quality of rice in relation to modifications in zinc dynamics. Available literature concerned to the present programme has been reviewed and presented as follows.

2.1 IMPACT OF ELEVATED CO_2 ON CROP GROWTH AND DEVELOPMENT:

The climatic variability and the predicted climatic changes are of major concern to the rice crop researchers because of their potential threat to rice productivity and the associated effect on the socioeconomic structure of many rice-growing countries. Amid the global atmospheric changes, the increasing concentrations of greenhouse gases such as CO_2 may have significant effect on rice productivity due to increase in the average surface temperature and also the amount of CO_2 available for photosynthesis (Aggarwal, 2003).

Increased photosynthesis that which leads to vigorous plant growth and higher yield is a common denominator of plants grown at eCO_2 (McDonald *et al*., 1999; Tognetti and Johnson, 1999; Franzaring *et al*., 2011; Tausz *et al*., 2013).

Several researchers have reported that enhancement in atmospheric CO_2 concentration increases crop growth, development and yield (Kimball 1983; Poorter and Perez-Soba, 2001; Leakey, 2009).

The major physiological processes which are responsible for the increase in crop production under eCO_2 is due to the increase in photosynthetic rate of crops (Bowes, 1993; Drake *et al*., 1997; Makino and Mae, 1999).

Besides this some growth parameters like leaf area, number of tillers and the shoot-root ratio also changes with the increase in atmospheric CO_2 concentration (Jitla *et al*., 1997; Masle 2000; Seneweera and Conroy 2005). Rise in atmospheric

CO_2 concentration leads to improvement in the growth of C_3 crops like wheat, rice and soybean than crops with C_4 photosynthetic pathway like maize, sorghum, and sugarcane (Myers *et al.*, 2014).

Increase in CO_2 level leads to increased carboxylation rates and decreases oxygenation rates of Rubisco enzyme in C_3 crop plants, which leads to greater photosynthesic rate and produces higher amount of carbohydrates (Stitt 1991; Drake *et al.*, 1997).

Elevated CO_2 stimulates the root growth of rice (Kim *et al.*, 2001). Seneweera (2011) reported that eCO_2 increased biomass at tillering stage and this was largely due to an increase in root mass by 160%.

According to Nakagawa and Horie (2000), a doubled CO_2 level increased rice biomass by 25%. Horie *et al.* (2000) mentioned an increase in rice yield by 30% with doubled CO_2 concentration.

A study conducted by Kim *et al.* (2001) revealed that yield has increased in rice under eCO_2 whereas increase in grain yield was lesser than that of increase in total biomass yield which resulted in the decrease in HI. An increase in radiation use efficiency (RUE) of rice was found with CO_2 enrichment relative to ambient CO_2. Elevated CO_2 has enhanced the light-saturated photosynthetic rate and apparent quantum yield of rice (De Costa *et al.*, 2006). According to DaMatta *et al.* (2009), rice grown eCO_2 has shown more vegetative growth compared to ambient level of CO_2
and Open fields.

In a FACE study in rice, under eCO_2 condition (570 ppm), there was an increase in the number of panicles per unit area (8%), number of spikelets per panicle (10%) and grain biomass (4%), which has led to the enhanced grain yield (Yang *et al.*, 2009).

FACE studies have shown an increase in yield by 7% to 15% at 570 µmol mol^{-1} CO_2 concentration for the Indica rice varieties (Kim *et al.*, 2003 a, b). Japonica rice cultivars also have shown similar responses to eCO_2 levels (Hatfield *et al.*, 2011).

2.2 EFFECT OF ELEVATED CO_2 ON GROWTH AND DEVELOPMENT OF RICE:

2.2.1 Plant height:

Elevated CO_2 (570 µmol mol^{-1}) strongly increased plant height in FACE experiment of rice crop (Liangyoupeijiu) (Yang *et al.*, 2009). Plant height at the time of harvest was higher under eCO_2 by 6.1%.

2.2.2 Number of tillers:

The eCO_2 (560 µmol mol^{-1}) enhanced both total number of tillers and productive tillers in rice (Akihikari) by using a temperature gradient type CO_2 concentration control chamber (TGC) (Kim, 1996).The number of tillers was enhanced at the maximum tillering stage by 42% due to the exposure of plants to eCO_2 (700 µmol CO_2 L^{-1}) in rice (*Oryza sativa* L. cv Jarrah) under growth chambers (Jitla *et al.*, 1997).

Various controlled environment experiments have shown that eCO_2 increased no.of tillers and panicles in rice (Ziska *et al.*, 1997; Baker and Allen, 1993; Moya *et al.*, 1998; Kim *et al.,* 2003a).

The number of tillers increased under eCO_2 (650 µmol mol^{-1}) at maximum tillering stage by 22% (45 DAT) and at the time of harvest by 8% (Sakai *et al.,* 2001). De Costa's experiment indicated that the tiller number/hill was higher (avg. 12 nos.) under eCO_2 (700 µmol mol^{-1}) compared to ambient CO_2 (avg. 10 no.) and open field (avg. 9 no.) in Maha Season of 2001 (De Costa *et al.,* 2003).

The number of tillers m^{-2} at heading stage was increased under eCO_2 (570 ± 35 µmol mol^{-1}) for all tested cultivars by 1-24% (Shimono *et al.,* 2009). In this experiment Kirana 397 and Hitomebore (early maturity varieties) has shown an enhancement in number of tillers by 22% and 18% respectively followed by the intermediate maturity varieties Akitakomachi (10%) and Kakehashi (4%) under FACE experiment.

2.2.3 Duration to flowering:

Several reports on agricultural crops have shown a positive impact of eCO_2 on growth and yield. This positive effect was due to a longer vegetative phase caused a delay in flowering time under eCO_2, e.g., in rice (Shimono *et al.,* 2009).

The effect of eCO_2 (680 ppm) on a grassland ecosystem using a FACE facility in California has shown a delay in flowering time by 2-6 days (Cleland *et al.*, 2007). The flowering time was delayed by 7 days in rice when exposed to eCO_2 (700±20 µL CO_2 L^{-1}) (Jagadish *et al.*, 2016).

2.3 IMPACT OF ELEVATED CO2 ON YIELD ATTRIBUTES OF RICE:

2.3.1 No. of grains per panicle:

An increase of 20% in the number of grains per panicle was indicated by Jilta et al. (1997) under eCO_2 (100 Pa). In Srilanka, the number of grains per panicle was higher (18%) in Maha season under OTC in eCO_2 (570 µmol mol^{-1}) than in ambient CO_2 (11% increase over open field) and open field (De Costa *et al.*, 2003). Under FACE experiment, the number of grains per panicle was enhanced at eCO_2 (680 ppm) at low night temperature of 22°C by 19% and decreased at by 82% high night temperature (32°C) at ambient CO_2 level and by 20% in open field.

The number of grains per panicle was enhanced by 9.6% in 2012 and by 6.8% in 2014 under eCO_2 (200µmol mol^{-1} above ambient condition) (Zhu *et al.*, 2015). No. of grains per panicle was increased in Takanari variety when averaged over years (2012, 2013, 2014) under eCO_2 (578 µmol mol^{-1}) by 8% but remained same in Koshihikari rice variety (Hasegawa *et al.*, 2019).

Similarly, other authors have also found similar results which have shown increased number of grains per panicle under eCO2 conditions (Manderscheid *et al.*, 1995; Hamid *et al.*, 2003).

2.3.2 Root weight:

The root weight (g m^{-2}) was enhanced among various cultivars grown under eCO2 (560 ± 26.3 µmol mol^{-1}) (Sakai *et al.*, 2019).

Higher root weight was observed in rice grown in eCO2 (622ppm) (41.5 g pot^{-1}) compared to to ambient CO2 (380ppm) (21.2 g pot^{-1}) (Kim *et al.*, 2011).

2.3.3 Straw yield:

An increase in straw yield (kg ha^{-1}) was observed in rice when cultivated under eCO2 in a FACE facility (499 µmol L^{-1}) (6509) compared to ambient (6137) and open field (5590) conditions (Satapathy *et al.*, 2015).

Increase in shoot weight was greater under eCO2 (622ppm) resulting in an increase of 46.6% compared with ambient CO2 (380ppm) (Kim *et al.*, 2011).

The shoot weight of rice crop (Yangdao 6 Hao) was increased by 29% grown under eCO2 in FACE experiment (ambient + 200 µmol mol^{-1}) relative to ambient CO2 (Zhu *et al.*, 2015).

2.3.4 Dry matter accumulation:

Rice (*Oryza sativa* L. subsp. Japonica cv. Dongjinbyeo) grown under eCO2 (622 ppm) six natural sunlit temperature gradient chambers (TGCs) has stimulated the dry matter accumulation (163.6g pot^{-1}) relative to ambient CO2 (380 ppm) (114.5g pot^{-1}) (Kim *et al.*, 2011).

Cheng *et al.* (2009) also observed that there was a similar significant increase in the whole plant dry weight at the time of harvest under eCO2 (680 ppm).

Total biomass (g m^{-2}) in a FACE facility across the years and varieties (Aikoku (released in 1882), Norin 8 (1934), Koshihikari (1956), Akihikari (1976) and Akidawara(2009)) was increased under eCO_2 (560 ± 26.3 µmol mol^{-1}) by 15.9% at maturity relative to ambient CO_2 (Sakai *et al*., 2019).

Madan *et al.* (2012) reported that the effect of eCO_2 (760 ppm) on total biomass was increased by 50% which was similar to other studies conducted in controlled environments (Baker, 2004; Ainsworth *et al.,* 2008).

The grain yield of Koshihikari and Takanari varieties was increased by 10%under eCO_2 (578 µmol mol^{-1}) compared to ambient CO_2 (378 µmol mol^{-1}) (Hasegawa *et al*., 2019).

2.4 IMPACT OF ELEVATED CO_2 ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF RICE:

2.4.1 Photosynthetic rate:

Net photosynthetic rate and gross photosynthetic rate in rice was enhanced by 8.9% and 11.3%, respectively under eCO_2 (667 ± 36 µmol mol^{-1}) (Sakai *et al*., 2001). Leaf net photosynthesis rate was increased under eCO_2, but its increased rate gradually slowed down through the growing season.

An increase in light-saturated photosynthetic rate (P_{max}) was noticed which was from 2% to 186% in Yala and in Maha seasons it was from 93% to 320% under eCO_2 (570 ± 42 µmol mol^{-1}) (De Costa *et al*., 2006). Improvement was observed in most of the varieties in both the seasons recorded an average increase of 58% computed by (Drake *et al*., 1997).

Regression model which evaluated net photosynthetic rate for Photosynthetic photon flux density of 1600 µmol m^{-2} s^{-1} at 350 and 700 µmol mol^{-1} noticed that rise in CO_2 across this range resulted in an increase in net photosynthetic rate by 17.4% (Baker *et al.,* 2005). P_{max} of leaf was greater at eCO_2 (548 ± 27 µmol mol^{-1})

particularly during vegetative growth stage (Shimono *et al.*, 2008). Photosynthetic rate was increased in the varieties Kirara397, Akitakomachi and Hitomebore by 36%, 35% and 48% respectively.

A number of field studies have revealed that rise in CO_2 has lead to an enhanced growth and increased productivity in rice cultivation due to increased net leaf photosynthesis (Sakai *et al.*, 2001; Roy *et al.*, 2012; Cai *et al.*, 2016, 2018).

On the other hand, the projected rise in atmospheric temperature may offset the benefits of eCO_2 and cause a reduction in net photosynthesis due to increased photorespiration and respiration, which inhibits dry matter accumulation (Ziska *et al.*, 1996; Dong *et al.*, 2001; Heskel *et al.*, 2016; Cai *et al.*, 2016).

2.4.2 Transpiration rate:

Transpiration rate observed in rice during panicle initiation stage was decreased under eCO_2 (800ppm) (5.851mmol H_2O m^{-2} s^{-1}) in green house compared to ambient CO_2 (400ppm) (6.517 mmol H_2O m^{-2} s^{-1}) (Rho *et al.*, 2020).

2.4.3 Stomatal conductance:

Stomatal conductance (gs) affects CO_2 diffusion from atmosphere to sub-stomatal cavity and is considered as an important factor determining the rate of photosynthesis and plant carbon metabolism. A decrease in gs is often noticed under eCO_2 in rice and many other species (Ainsworth and Rogers, 2007; Shimono *et al.*, 2010; Cai *et al.*, 2018). Some researchers claimed that warming increased gs (Mott and Peak, 2013; Urban *et al.*, 2017), while others have found stomatal closure is due to warming and that high temperature had no effect on gs (Sage and Sharkey, 1987).

However, as eCO_2 and temperature together affect plant carbon metabolism, a better understanding of their combined effects on gs is needed. At present, only one report i.e., Cai *et al.* (2018) showed that the combined effects of eCO_2 and temperature on gs were variable among different growth stages and varieties for field-grown rice.

2.4.4 Total chlorophyll:

The total chlorophyll content observed in rice during panicle initiation stage was increased in green house under eCO_2 (800ppm) [41.57(SPAD)] compared to ambient CO_2 (400ppm) [40.75 (SPAD)] (Rho *et al.*, 2020).

2.4.5 Total soluble protein:

Soluble protein content has shown a significant difference in rice variety CO 51 due to a different environmental condition. The increase in total soluble protein content was observed from tillering stage to the heading stage and a gradual decrease towards the grain filling stage. The highest soluble protein content (5.07, 8.03 and 7.11 mg g^{-1}) was noticed in ambient condition at 30, 60 and 85 DAT followed by OTC + ambient CO_2 (4.95, 7.29 and 6.74 mg g^{-1}) and the lowest content was registered at OTC + elevated CO_2 (4.52, 6.98 and 6.24 mg g^{-1}) (Vinothini *et al.*, 2019).

2.4.6 Carbohydrate:

The environmental condition had a significant influence on total carbohydrate content at different growth stages of the rice variety CO 51. Among the environmental conditions, the highest carbohydrate content was noticed in OTC + elevated CO_2 (52.6, 69.6 and 54.6 mg g^{-1}) followed by OTC + ambient CO_2 (42.4, 54.6 and 46.3 mg g^{-1}), while the lowest content was in ambient condition (32.8, 49.5 and 38.7 mg g^{-1}) at 30, 60 and 85 DAT (Vinothini *et al.*, 2019).

2.5 IMPACT OF ELEVATED CO_2 ON NUTRITIONAL QUALITY OF RICE:

Increasing concentration of atmospheric CO_2 lowers the content of zinc and other nutrients in important food crops such as rice, wheat, maize and barley (Myers *et al.*, 2015).

An effect of higher levels of CO_2 diminishes the elements such as zinc and iron which are already deficient in half of the global population (Loladze, 2002). Lieffering *et al.* (2004) has grown rice under FACE facility with standard field conditions and found that there is no effect of eCO_2 on nutrient content.

There were several other reports of decrement in zinc, iron, and protein in wheat, barley, and rice grown in outdoor open topped chambers or in indoor climate-controlled growth chambers (Dietterich *et al*., 2015).

2.6 IMPACT OF ELEVATED CO_2 ON ZINC DYNAMICS IN RICE:

Boonchuay *et al.* (2013) reported that maximum zinc content in grain under 0.5% $ZnSO_4$ foliar spray. In rice, foliar application enhanced content of Zn in grain (Yuan *et al*., 2013). The effectiveness was more with foliar application compared to soil applications (Yilmaz *et al*., 1997; Cakmak *et al.,* 2010).

In a FACE experiment with eCO_2 at 500±20 ppm, the zinc content in grain was 5.8 ppm when treated with 0.6g N pot^{-1}; 0.4g P_2O_5 pot^{-1}; 0.4g K_2O pot^{-1} whereas under ambient CO_2 (395 ppm) the zinc content was 5.2 ppm with same treatment (Raj, 2014).

Total zinc concentration (mg kg^{-1}DW) was also increased in stem and leaves at grain filling stage in IR68144 rice genotype. The highest accumulation of zinc was observed in shoot followed by leaves (Wu *et al*., 2010). Zinc accumulation increased in plant shoot with Zn-EDTA at 1.07 g L^{-1} of Zn (Alvarez *et al*., 2019). Foliar Zn supply with 0.5% $ZnSO_4$ enhanced the Zn concentration of the new young leaves which were developed after foliar Zn spray (Phuphong *et al*., 2020).

Barea (1991) reported that AMF can survive in waterlogged conditions and that *Glomus etunicatum* showed fairly high colonization in rice roots and best survival under submerged conditions (Purakayastha and Chhonkar, 2001). A meta-analysis validated that AMF positively affects Zn concentration in various plant tissues inclusive of rice (Lehman *et al*., 2014). The AMF inoculated rice plants (Transplanting of pre-mycorrhized seedlings) at eCO_2 (500 ppm) has enhanced the zinc concentration in grains by 61% when compared to uninoculated plants (Sahoo, 2017).

Seed priming with $ZnSO_4$ 0.004M (6.4%) for 36 hr enhanced the zinc content in rice grain (Johnson *et al*., 2005).

Materials &
Methods

3. MATERIALS AND METHODS

The study entitled "Influence of elevated CO_2 on zinc dynamics in rice (*Oryza sativa* L.)" was conducted in the Department of Plant Physiology, College of Agriculture, and Vellayani during 2018-20 with the objective to assess the impact of elevated CO_2 on growth, development and nutritional quality of rice in relation to modifications in Zn dynamics. The particulars of the materials used, methods acquired for the experiment and protocols followed during the course of experimentation are given a description in this chapter.

3.1 EXPERIMENTAL DETAILS

3.1.1 Location

The experiments were conducted in Open Top Chamber (OTC) and in low cost polyhouse located at College of Agriculture Vellayani, situated at 8°5' N latitude and 76°9' E longitude and an altitude of 29 m above mean sea level. (Plate 1. and 3.).

3.1.2 Planting material

Medicinal rice variety Njavara (golden yellow) was procured from Regional Agricultural Research Station, Pattambi, Palakkad district, Kerala. Another popular Kerala rice variety Uma (MO16) was collected from Integrated Farming Research Institute, Karamana, Thiruvananthapuram district, Kerala.

3.1.3 Layout of the Experiment

The experiment was laid out in CRD with 24 treatment combinations and three replications and with one pot per replication.

3.1.4 Treatments:

Conditions:

C_1- Open field condition (control)

C_2- Poly house

C_3- Open Top Chamber (OTC) with elevated CO_2 concentration (500 ppm)

Variety:

V_1- Uma (MO16)

V_2- Njavara - golden yellow

Treatments related to zinc dynamics:

D_1- Foliar spray ($ZnSO_4$ 0.5% at panicle initiation and grain filling stage).

D_2- AMF (3g of AMF inoculum /cavity of protrays)

D_3- Seed treatment: seed priming with 6.4% $ZnSO_4$ for 36 hrs.

D_4- No treatment

Design: CRD

Replications: 3

Treatment combinations: 3x2x4=24

No. of pots per treatment: 6

Plate 1. Open Top Chamber for CO_2 enrichment

Plate 2. Various sensors and CO_2 cylinders used in Open Top Chamber

Plate 3. Low-technology Polyhouse

3.2 EXPERIMENT

Determining the impact of elevated CO_2 on growth, development and quality of Rice

3.2.1 Technique for CO_2 enrichment

The main system used for subjecting the plants to elevated CO_2 (eCO_2) environment is an Open Top Chamber, maintained by the Department of Plant Physiology at College of Agriculture, Vellayani. For the present programme CO_2 concentration of 500 ppm was maintained within this chamber.

The facility of Open Top Chamber system consists of square type chambers constructed to maintain eCO_2 conditions for experimental studies. The basic structure of OTC was built of metal frame covered with a 200 micron UV poly sheet. The chamber was 3 X 3 X 3 dimension, 45° slope and $1m^2$ opening at the top. CO_2 was released into the chamber from a CO_2 cylinder in a controlled manner. Measurement of microclimatic parameters (temperature, humidity and light) was done inside and outside the OTC with the help of sensors on a real time basis (Plate2.). Mean temperature of 38°C and relative humidity of 82.60 % were recorded inside chamber during the experimental period.

The elevated CO_2 concentration of 500 ppm was opted on the basis of IPCC (2007) which suggested that atmospheric concentrations of CO_2 have been steadily rising with an average annual increase rate of 2 ppm and continue to rise 500- 1000 ppm by the year 2100.

Other conditions:

A low cost polyhouse was used for the second level of CO_2 concentration. The average CO_2 concentration and mean air temperature in the polyhouse was 420 ppm and 36°C respectively and in the open field condition it was 390 ppm and 33.2°C.

3.2.2 Methodology

D_1 - Foliar spray

The seeds of both Uma (MO16) and Njavara-golden yellow varieties were soaked in distilled water for 24hrs. Soaked seeds were placed in petriplates for 3 days for germination. The germinated seeds were grown in protrays were transplanted to pots. Foliar spray with $ZnSO_4$ (0.5%) was given at panicle initiation stage and grain filling stage for the experimental plants kept under all the three CO_2 conditions.

D_2 - Treatment with AMF

The seeds of both the varieties were soaked in distilled water for 24hrs and were placed in petriplates for 3 days for germination. Three grams of AMF inoculum was applied in each cavity of protrays. The germinated seeds were grown in protrays for 20 days. Then the seedlings were transplanted to pots and were maintained till harvest.

D_3 - Seed priming

Seeds of both the varieties were immersed in 6.4% $ZnSO_4$ for 36 hrs. Soaked seeds were placed in petriplates for 3 days for germination.The germinated seeds were grown in protrays and then the 20 days old rice seedlings were transplanted to pots.

D_4 - No treatment

The seeds of two rice varieties were soaked in distilled water for 24hrs. Soaked seeds were placed in petriplates for 3 days for germination. The germinated seeds were grown in protrays (20 days). Then the rice seedlings were transplanted to pots.

All the pots used for the experiment were filled with potting mixture by mixing soil, sand and FYM in the ratio of 1:1:1. The protrays used were of 100 cell size and were filled with coir pith and vermicompost in 1:1 ratio.

Foliar application of 19:19:19 was given 2 weeks after transplanting. Experimental plants were maintained as per package of practices of Kerala Agricultural University, Thrissur. The plant protection measures were carried out as per *ad hoc* recommendations of Kerala Agricultural University, Thrissur (KAU, 2011).

Plate 4. Steps followed for the preparation of planting material

Uma (MO 16)

Njavara- golden yellow

Soaked in distilled water for 24 hrs

Soaked in 6.4% $ZnSO_4$ for 36 hrs

Uma

Njavara

Uma

Njavara

3 days for germination

Germinated seeds were sown in protrays containing vermicompost and coirpith (1:1)

Seedlings of Uma (2 weeks after sowing) with seed treatment (6.4% $ZnSO_4$)

Seedlings of Njavara (2 weeks after sowing) with seed treatment (6.4% $ZnSO_4$)

For AMF treatment, 3g of AMF inoculum was mixed in each cavity of protrays

Seedlings of Uma (2 weeks after sowing) with AMF treatment

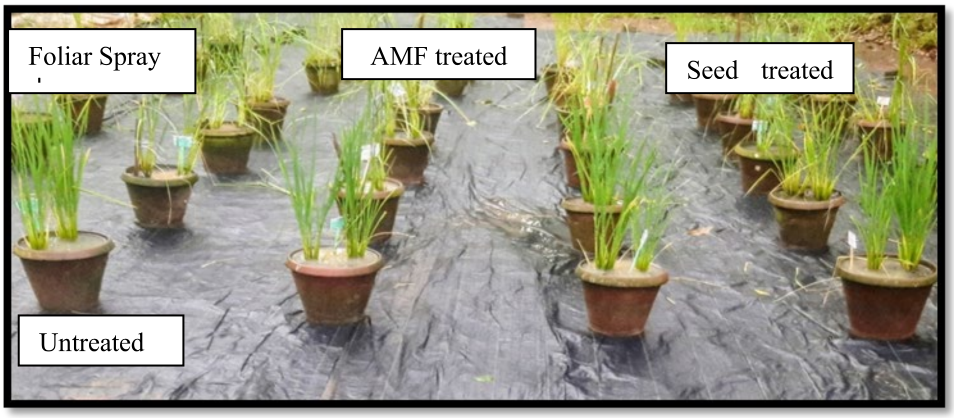
Seedlings of Njavara (2 weeks after sowing) with AMF treatment

After 20 days, the seedlings were transplanted to pots

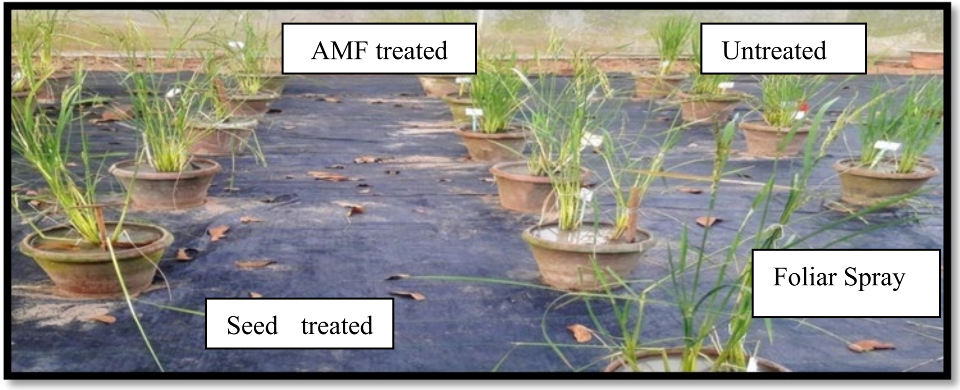
Plants were maintained as 3 sets for the different treatments

Plate 5. Overview of plants grown in open field condition

TransplantingStage

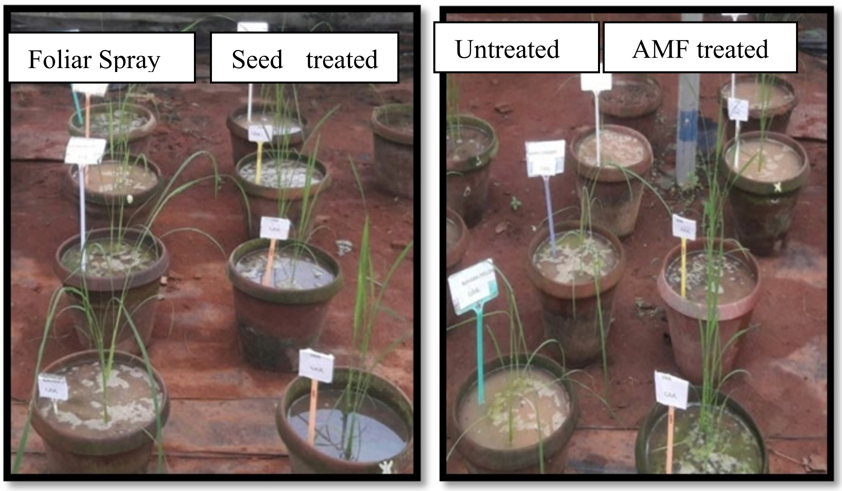


Tillering Stage

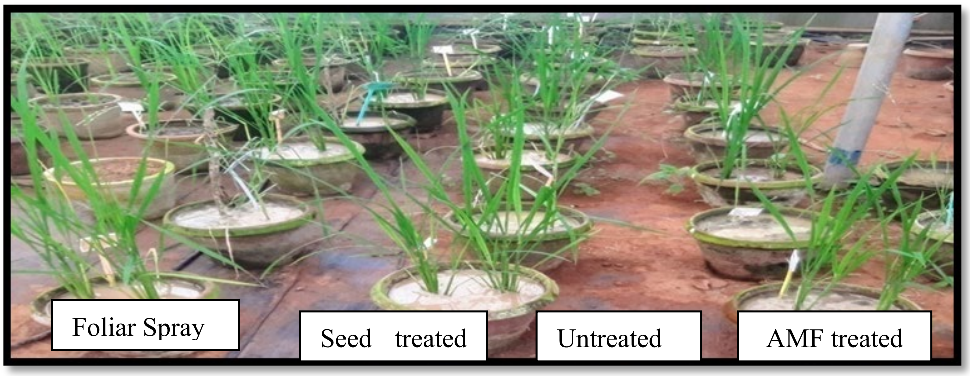


Maturity Stage

Plate 6. Overview of plants grown in Polyhouse



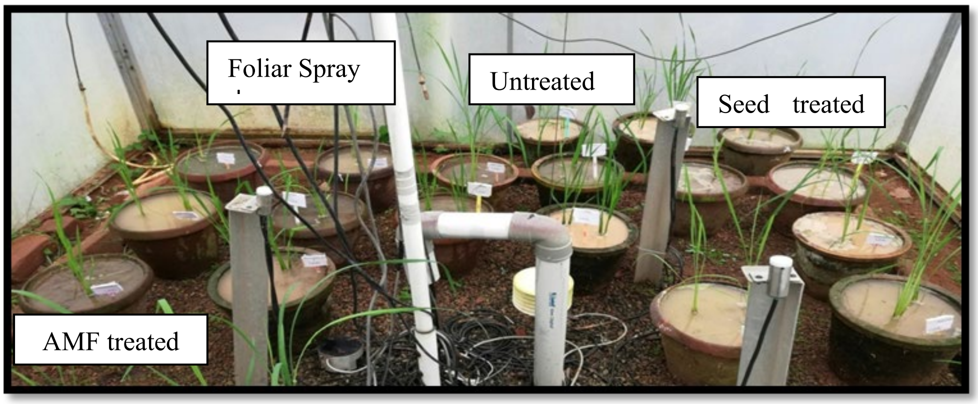
Transplanting Stage



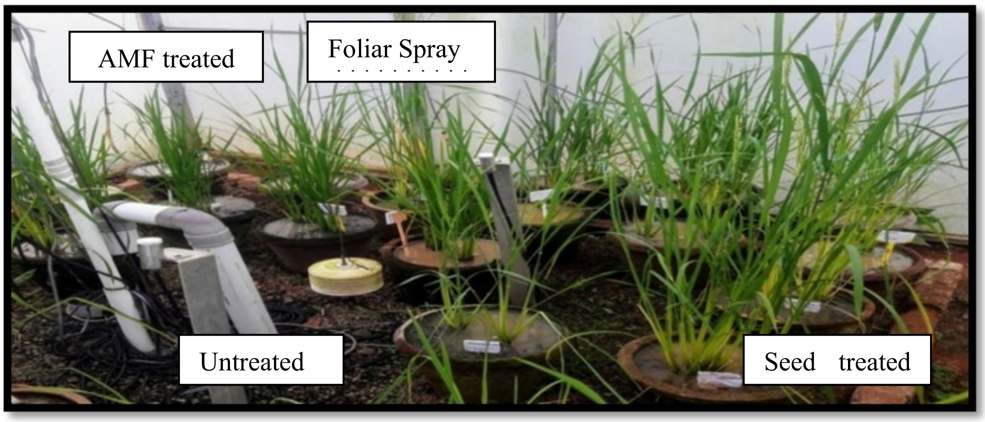
Tillering Stage

Flowering Stage

Plate 7. Overview of plants grown in OTC



TransplantingStage



Tillering stage

Flowering stage

After giving the specific treatment, one set of plants was transferred to OTC (Plate7.), a second set to polyhouse (Plate6.) and the third set (Plate5.) was retained under the ambient CO_2 where the average CO_2 concentration was 390 ppm. Plants were maintained under flooded condition till harvest. Observations on physiological and biochemical parameters were made at panicle initiation and grain filling stage and observations on morphological parameters and parameters related to zinc dynamics were made at the time of harvest.

3.2.3 Observations recorded

Observations on growth, physiological and biochemical parameters were recorded at 20, 45, 60, 90 DAT for Njavara- golden yellow and at 35, 60, 80, 120 DAT for Uma (MO16). Observations on nutrient status were made at 90 DAT for Njavara variety and at 120 DAT for Uma variety.

3.2.4 Morphological parameters

3.2.4.1 Growth parameters

3.2.4.1.1 Plant height

The plant height was recorded from the base of the plant to the top leaf of the main shoot and expressed in centimeter (cm).

3.2.4.1.2 Days to 50% flowering

Number of days taken for 50 % of the hills to flower from the date of sowing of the cultivars was recorded.

3.2.4.1.3 Root weight

Roots after removing all the adhering soil particles were washed thoroughly and were dried in a hot air oven at a temperature of 80ºC for 48 hours (till attaining a constant weight). Then the dry weight was recorded using an electronic balance in g/plant.

3.2.4.1.4 Number of tillers

The number of aerial shoots arising around a single plant was counted (total no. of plants-144).

3.2.4.1.5 Straw yield

The straw yield was recorded using an electronic balance in g/plant after drying the straw in hot air oven at 80ºC for 48 hours till reaching a constant weight.

3.2.5 Physiological and Biochemical parameters

3.2.5.2 Photosynthetic rate

Photosynthetic rate was recorded between 8:30 am and 11 am using portable photosynthetic system (CIRAS-3, PP systems U.S.A) and was expressed in µmoles $m^{-2} s^{-1}$.

3.2.5.4 Transpiration rate

Transpiration rate was recorded between 8:30 am and 11 am using portable photosynthetic system (CIRAS-3, PP systems U.S.A) and was expressed in mmoles H_2O $m^{-2} s^{-1}$.

3.2.5.3 Stomatal conductance

Stomatal conductance was recorded between 8:30 am and 11 am using a portable photosynthetic system (CIRAS-3, PP systems U.S.A) and was expressed in mmoles $m^{-2} s^{-1}$.

3.2.5.4 Total chlorophyll

Leaves collected from all the three CO_2 conditions were washed in distilled water and used for the estimation of chlorophyll. The leaf bits of 100 mg were incubated in 80 % Acetone: DMSO (Dimethyl sulfoxide (1:1 v/v) mixture in the dark for overnight. The chlorophyll extract was decanted into measuring cylinder and made up to 10 ml. The absorbance was recorded at 663 and 645 nm using UV-visible spectrophotometer. Total chlorophyll content was estimated as described by Sadasivam and Manickam (1996) and was calculated by using the following formula and expressed as mg g^{-1} of fresh weight.

Total chlorophyll = [{20.2 (A645) + 8.01 (A663)} × volume/ weight] × 1000

3.2.5.5 Total soluble protein

Total soluble protein content of leaves was estimated as described by He (2011) using bovine serum albumin as the standard. Sample of 0.5 g was homogenised in 2.5 ml of ice cold extraction phosphate buffer (pH- 7.8) containing 1 mM EDTA, 2% (w/w) and isolation was done at $4^{O}C$ on ice. Centrifuged at 15000g for 20 mins at $4^{O}C$. To the 100µl of the supernatant 3 ml of Bradford reagent was added and mixed well. The absorbance of the solution was recorded after 2 minutes and within 30 minutes using the BSA standard in the range of (10-100mg) at 595 nm using spectrophotometer. The protein content was expressed as mg g^{-1}.

3.2.5.6 Total Reducing sugars **(Dinitrosalicylic acid (DNS) method)**

Reducing sugar content of rice leaves was estimated as described by Sadasivam and Manickam (1996). 100mg of the sample was weighed and the sugars were extracted using hot 80% alcohol twice (5ml each time). Collected the supernatant and evaporated on water bath. 10ml of water was added to dissolve the sugars. Pipetted out 0.5 to 3 ml of alcohol-free extracted into test tubes and made up the volume to 3 ml with water in all the tubes. 3ml of DNS reagent was added and mixed. Later on heated for 5 minutes in a boiling water bath. After the colour has developed, 1 ml of 40% Rochelle salt solution was (when the contents are still warm) and mixed. The test tubes were cooled under running tap water and the absorbance was measured at 510 nm using reagent blank adjusted to zero absorbance. The amount of reducing sugar in the sample was calculated using a standard graph prepared from working standard glucose solution (0 to 500 µg) in the same manner.

3.2.6 Parameters related to zinc dynamics

3.2.6.1 Nutrient status

The leaf and shoot samples of both the varieties were collected from plants kept under open field, poly house and elevated CO_2 conditions. They were initially sundried and then oven dried to a constant weight. The dried samples were finally ground and sieved. Zn content was analysed by diacid extracts. This was made by digesting 1 g of the sample in 10 ml of 9:4 concentrated nitric acid and perchloric acid mixture. Aliquots of the digests were taken for the analysis.

Zn content in shoot and leaf was determined using Atomic Absorption Spectrophotometer (Analytik Jena company with novAA (300) model) at Onattukara Regional Agricultural Research Station, Kayankulam, Kerala and the content of nutrient was calculated and expressed in $\mu g\ g^{-1}$.

Statistical Analysis:

The analysis of variance (ANOVA) was carried out using OPSTAT software to assess the significance difference between the treatments and their interaction.

Results

4. RESULTS

The present investigation was designed to improve zinc status and to assess the growth and development in rice plants which has been decreasing due to the raise in CO_2 in the atmosphere with various zinc enrichment treatments which was done at OTC and Polyhouse situated in College of Agriculture, Vellayani. The rice plants were exposed to elevated CO_2 with 500 ppm concentration at OTC, Polyhouse with 420 ppm on an average and open field condition with ambient CO_2 at 390 ppm from September 2019 to January 2020.

The observations related to growth and development of rice plants was recorded and the nutrient status of rice plants was analyzed at different CO_2 concentrations. The analyzation of data was done statistically and the results are presented in this chapter with suitable tables.

4.1 OBSERVATIONS ON GROWTH PARAMETERS OF RICE AT DIFFERENT CO_2 CONCENTARTIONS AND UNDER ZINC TREATMENTS:

4.1.1 Plant Height (cm):

The control plants which were maintained without any treatment (D4) responded significantly to the different CO_2 levels in terms of plant height. Plant height was increasing with increasing CO_2 levels and the highest mean plant height was recorded under OTC condition (108cm) followed by those under Polyhouse (97cm) and control plants (89cm).

All the zinc enrichment treatments influenced plant height significantly under all the three CO_2 conditions. Among the treatments, D_1 (Foliar spray) has shown highest plant height followed by D_3 (Seed treatment), D_2 (AMF treatment) and D_4 (No treatment) in the under all the conditions. The highest value recorded was 102 cm [D_1 (Foliar spray)] in the case of Uma and 130cm [D_1 (Foliar spray)] cm in the case of Njavara under OTC. Plants kept under ambient condition without any treatment (D_4)

recorded lowest values, 72 cm in the case of Uma and 84.5cm in the case of Njavara. The results pertaining to plant height is presented in Table1.

Table 1. Effect of zinc treatments and CO_2 concentrations on plant height (cm) of rice:

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN (V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	89.7	86.0	83.3	72.0	97.3	91.0	84.7	78.3	102.0	100.2	95.7	88.3	89.0
Njavara	102.0	96.2	94.3	84.5	113.2	108.3	105.0	97.7	130.0	123.3	117.0	104.5	106.3
MEAN(C)	89				97				108				
	CD(0.05): D= 3.568,V= 2.523,C*D= N/A, V*C=4.369, D*V= N/A												
	SE±(m): D=1.254, V=0.887,C*D=2.173, V*C=1.536, D*V=1.774												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.1.2 Days to 50% flowering:

The number of days to 50% flowering has shown significant difference to different CO_2 levels when compared to the control plants without any treatment (D_4). The shortest number of days to 50% flowering was recorded in Open field condition (67.8) followed by those Polyhouse (75.5) and OTC (78.3).

The Zn treatments did not result in any significant difference in the time taken to reach 50% flowering.

The number of days to 50% flowering was delayed by 10-11 days under OTC and Polyhouse relative to Open field condition.

Rice variety Njavara (Yellow) is a short duration variety (80-90 days) whereas Uma is a medium duration variety (115-120 days). Njavara took minimum number of days to 50% flowering (60 days) under Open field condition followed 69 days under Polyhouse condition and 71 days under OTC. In the case of Uma, the minimum number of days to 50% flowering was 73 days under Open field condition followed by 80 days under Polyhouse and 85 days under OTC. The results pertaining to number of days to 50% flowering is presented in Table2.

Table 2. Effect of zinc treatments and CO_2 concentrations on days to 50% flowering of rice:

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN (V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	73.3	72.7	74.0	73.3	80.0	80.3	82.0	82.3	84.0	85.3	86.3	86.7	80.0
Njavara	62.7	61.7	62.7	61.7	69.3	69.0	70.3	70.7	71.3	70.7	71.7	70.3	67.7
MEAN (C)	67.8				75.5				78.3				
	CD(0.05): D= N/A,V= 0.770,C*D= N/A, V*C=1.334, D*V= N/A												
	SE±(m): D=0.383, V=0.271,C*D=0.663, V*C=0.469, D*V=0.541												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.1.3 No. of tillers:

The number of tillers has shown significant difference among the control plants kept under different CO_2 levels that were grown without any treatment (D_4). Highest number of tillers was recorded in OTC (30.1) followed by the plants kept in the Polyhouse (21.1) and control plants (18).

All the zinc enrichment treatments influenced no. of tillers significantly under all the three CO_2 conditions. Among the treatments, D_1 has resulted in highest number of tillers followed by D_3, D_2 and D_4 under all the conditions. The highest value recorded was 34 (D_1) in the case of Uma and 32 (D_1) in the case of Njavara under OTC. Plants cultivated under open field condition without any treatment (D_4) recorded lowest values, 17.7 in the case of Uma and 10.3 in the case of Njavara. The results pertaining to no. of tillers is presented in Table3.

Table 3. Effect of zinc treatments and CO_2 concentrations on No. of tillers of rice:

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	24.3	23.0	24.0	17.7	26.3	22.7	23.0	21.7	34.0	30.7	32.0	28.7	25.7
Njavara	17.7	14.0	13.3	10.3	20.7	18.0	20.0	16.7	32.0	29.3	29.7	24.3	20.5
MEAN(C)	18.0				21.1				30.1				
	CD(0.05): D=1.584, V=1.120,C*D= N/A, V*C=1.940, D*V= N/A												
	SE(m): D=0.557, V=0.394, C*D=0.965, V*C=0.682, D*V=0.788												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.1.4 Root weight (g /plant):

The control plants which were maintained without any treatment (D_4) responded significantly to the different CO_2 levels in terms of root weight. Root weight was increasing with increasing CO_2 levels and the highest root weight was recorded under OTC condition (19.1 g/plant) followed by those under Polyhouse (16.8 g/plant) and then by the control plants (14.2 g/plant).

All the zinc enrichment treatments influenced root weight significantly under all the three CO_2 conditions. Among the treatments, D_2 has shown highest root weight followed by D_3, D_1 and D_4 under all the conditions. The highest value recorded was 26.7 g/plant (D_2) in Uma and 20.4 g/plant (D_2) in Njavara under OTC. Plants kept under Open field condition without any treatment (D_4) recorded lowest values, 10.9 g/plant in the case of Uma and 7.4 g/plant in the case of Njavara. The results pertaining to root weight is presented in Table4.

Table 4. Effect of zinc treatments and CO_2 concentrations on root weight (g/plant) of rice:

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	15.4	22.0	19.4	10.9	18.7	24.9	21.8	14.2	22.0	26.7	24.3	17.3	19.8
Njavara	10.6	15.3	12.4	7.4	11.9	18.3	14.8	9.7	14.1	20.4	17.4	10.8	13.6
MEAN(C)	14.2				16.8				19.1				
	CD(0.05): D=1.115,V= 0.788,C*D= N/A, V*C= N/A, D*V= N/A												
	SE±(m): D=0.392, V=0.277,C*D= 0.679, V*C=0.480, D*V=0.554												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.1.5 Straw yield (g/plant):

The straw yield was significantly influenced by different CO_2 levels. Highest straw yield was recorded by the experimental plants kept in OTC (36.6 g/plant) followed by those in Polyhouse (27.5 g/plant) and then by the control plants (22.2 g/plant).

All the zinc enrichment treatments influenced straw yield significantly under all the three CO_2 conditions. Among the treatments, D_1 has shown highest straw yield followed by D_3, D_2 and D_4 under all the conditions. The highest value recorded was 35 g/plant (D_1) in Uma and 46.8 g/plant (D_1) in Njavara under OTC. Plants kept under Open field condition without any treatment (D_4) recorded lowest values, 17.5 g/plant in the case of Uma and 22.9 g/plant in the case of Njavara. The results pertaining to straw yield is presented in Table5.

Table 5. Effect of zinc treatments and CO_2 concentrations on straw yield (g/plant) of rice:

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	23.2	18.8	20.3	17.5	29.7	24.7	26.7	21.5	35.0	29.3	29.0	27.3	25.2
Njavara	27.3	23.2	24.4	22.9	32.5	28.9	30.7	25.5	46.8	43.5	44.3	38.0	32.3
MEAN(C)	22.2				27.5				36.6				
	CD(0.05): D=0.631,V= 0.446,C*D= 1.093, V*C=0.773, D*V=0.893												
	SE±(m): D=0.222, V=0.157,C*D=0.384, V*C=0.272, D*V=0.314												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2 OBSERVATIONS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF RICE AT DIFFERENT CO_2 CONCENTARTIONS AND UNDER ZINC TREATMENTS:

4.2.1 Total chlorophyll (active tillering stage) (mg g^{-1} fresh weight):

The total chlorophyll content of leaves at active tillering stage was significantly high under elevated CO_2 levels relative to the control plants that were maintained in ambient condition. Highest chlorophyll content was recorded in plants under Polyhouse condition (2.33 mg g^{-1} fresh weight) followed by those under OTC (2.18 mg g^{-1} fresh weight) and control plants (2.06 mg g^{-1} fresh weight).

Among the treatments, D_1 has shown highest chlorophyll content in Uma (1.57 mg g^{-1} fresh weight) and in Njavara also D_1 has shown highest chlorophyll content under Polyhouse (2.89 mg g^{-1} fresh weight). The lowest chlorophyll content was observed in untreated plants under open field condition i.e., 1.46 mg g^{-1} fresh weight in Uma and 2.28 mg g^{-1} fresh weight in Njavara. The results pertaining to total chlorophyll content at active tillering stage is presented in Table6.

Table 6. Effect of zinc treatments and CO_2 concentrations on total chlorophyll content of rice at active tillering stage (mg g^{-1} fresh weight):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	1.56	1.55	1.54	1.46	1.57	1.55	1.52	1.49	1.55	1.50	1.50	1.49	1.56
Njavara	2.38	3.07	2.57	2.28	2.89	3.81	2.91	2.91	3.12	2.66	3.04	2.60	2.38
MEAN(C)	2.05				2.33				2.18				
	CD(0.05): D= 0.108,V= 0.076,C*D= 0.186, V*C=0.132, D*V= 0.152												
	SE±(m): D=0.038, V=0.027,C*D=0.066, V*C=0.046, D*V=0.054												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.2 Total chlorophyll (grain filling stage) (mg g^{-1} fresh weight):

The total chlorophyll content of leaves at grain filling stage has shown significant difference at different CO_2 levels by the control plants. Highest chlorophyll content was observed in plants kept in OTC (1.805 mg g^{-1} fresh weight) followed by those in the Polyhouse (1.803 mg g^{-1} fresh weight) and then by the open field condition (1.684 mg g^{-1} fresh weight).

Among the treatments, D_1 has resulted in highest chlorophyll content in Uma (1.52 mg g^{-1} fresh weight) and in Njavara also plants receiving D_1 has shown highest chlorophyll content (2.25 mg g^{-1} fresh weight) under OTC. The lowest chlorophyll content was recorded in untreated plants under open field condition i.e., 1.40 mg g^{-1} fresh weight in Uma and 1.85 mg g^{-1} fresh weight in Njavara. The results pertaining to total chlorophyll content at grain filling stage is presented in Table7.

Table 7. Effect of zinc treatments and CO_2 concentrations on total chlorophyll content of rice at grain filling stage (mg g^{-1} fresh weight):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	1.44	1.42	1.43	1.40	1.51	1.51	1.48	1.42	1.52	1.51	1.50	1.48	1.47
Njavara	1.80	2.13	2.00	1.85	2.09	2.24	2.17	2.01	2.25	2.00	2.11	2.07	2.06
MEAN(C)	1.684				1.803				1.805				
	CD(0.05): D=0.035, V=0.025,C*D=0.060, V*C=0.042, D*V=0.049												
	SE(m): D=0.012, V=0.009, C*D=0.021, V*C=0.015, D*V=0.017												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.3 Total soluble protein (active tillering stage) (mg g^{-1}):

There was significant difference among the open field condition plants which were maintained without any treatment (D_4) under different CO_2 levels in terms of total soluble protein at active tillering stage. Highest total soluble protein content was recorded in Open field condition (3.1 mg g^{-1}) followed by Polyhouse (1.1 mg g^{-1}) and OTC (0.8 mg g^{-1}) systems.

Among the treatments, D_1 has shown highest chlorophyll content in Uma (3.15 mg g^{-1}) and in Njavara also D_1 has shown highest total soluble protein content under Open field condition (3.13 mg g^{-1}). The lowest total soluble protein content was recorded in untreated plants under OTC i.e., 0.65 mg g^{-1} fresh weight in Uma and 0.76 mg g^{-1} fresh weight in Njavara. The results pertaining to total soluble protein at active tillering stage is presented in Table8.

Table 8. Effect of zinc treatments and CO_2 concentrations on total soluble protein content of rice at active tillering stage (mg g^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN (V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	3.18	3.10	3.15	3.06	1.30	1.19	1.12	0.96	0.86	0.72	0.80	0.65	1.67
Njavara	3.15	3.10	3.13	3.04	1.21	1.11	1.12	1.10	0.92	0.81	0.90	0.76	1.69
MEAN(C)	3.1				1.1				0.8				
	CD(0.05): D=0.042,V= N/A,C*D= 0.072, V*C=0.051, D*V= N/A												
	SE±(m): D=0.015, V=0.010,C*D=0.025, V*C=0.018, D*V=0.021												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.4 Total soluble protein (grain filling stage) (mg g^{-1}):

There was significant difference among the control plants (D_4) kept under different CO_2 levels in terms of total soluble protein at grain filling stage. Highest total soluble protein content was recorded in Open field condition (7.1 mg g^{-1}) followed by Polyhouse (5.9 mg g^{-1}) and OTC (4.0 mg g^{-1}).

All the zinc enrichment treatments have responded significantly under all the three CO_2 conditions. Among the treatments, D_1 has shown excessive total soluble protein content in Uma (7.03 mg g^{-1}) and in Njavara also D_1 has shown highest total soluble protein content under Open field condition (7.14 mg g^{-1}). The lowest total soluble protein content was recorded in untreated plants under OTC i.e., 3.67 mg g^{-1} fresh weight in Uma and 3.79 mg g^{-1} fresh weight in Njavara. The results pertaining to total soluble protein at grain filling stage is presented in Table9.

Table 9. Effect of zinc treatments and CO_2 concentrations on total soluble protein content of rice at grain filling stage (mg g^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	7.12	7.03	7.07	7.06	6.09	5.50	5.87	5.85	4.04	3.91	4.03	3.67	5.60
Njavara	7.14	6.92	7.14	7.05	6.06	5.65	6.00	5.90	4.30	3.91	4.01	3.79	5.66
MEAN(C)	7.1				5.9				4.0				
	CD(0.05): D=0.151,V= N/A,C*D= N/A, V*C= N/A, D*V= N/A												
	SE±(m): D= N/A, V= N/A,C*D=0.092, V*C=0.065, D*V=0.075												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.5 Total reducing sugars (active tillering stage) (mg g^{-1}):

The total reducing sugars of leaves at active tillering stage was significantly high under elevated CO_2 levels relative to the control plants that were maintained in ambient condition. The highest total reducing sugar content was recorded in OTC (1.9 mg g^{-1}) followed by Polyhouse (1.3 mg g^{-1}) and then by the control plants (1.2 mg g^{-1}).

All the zinc enrichment treatments have responded significantly under all the three CO_2 conditions. Among the treatments, D_1 has shown highest total reducing sugar content in Uma (2.36 mg g^{-1}) and in Njavara also D_1 has shown highest reducing sugar content under OTC (2.25 mg g^{-1}). The lowest value was recorded in untreated plants under Open field condition i.e., 1.13 mg g^{-1} in Uma and 1.1 mg g^{-1} in Njavara. The results pertaining to total reducing sugars of leaves at active tillering stage is presented in Table10.

Table 10. Effect of zinc treatments and CO_2 concentrations on total reducing sugars of rice at active tillering stage (mg g^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	1.28	1.17	1.18	1.13	1.52	1.21	1.24	1.64	2.36	1.92	2.01	1.85	1.54
Njavara	1.22	1.15	1.18	1.1	1.52	1.19	1.22	1.15	2.25	1.27	1.67	1.65	1.38
MEAN(C)	1.2				1.3				1.9				
	CD(0.05): D=0.084,V= 0.059,C*D= 0.145, V*C=0.103, D*V= N/A												
	SE±(m): D=0.030, V=0.021,C*D=0.051, V*C=0.036, D*V=0.042												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.6 Total reducing sugars (grain filling stage) (mg g^{-1}):

The total reducing sugars of leaves at grain filling stage was significantly high under elevated CO_2 levels relative to the control plants that were maintained in ambient condition. The highest total reducing sugar content was recorded in OTC (6.3 mg g^{-1}) followed by those in Polyhouse (4.3 mg g^{-1}) and then by the control plants (2.9 mg g^{-1}).

All the zinc enrichment treatments had significant effects under all the three CO_2 conditions. Among the treatments, D_1 resulted in highest total reducing sugar content in Uma (6.36 mg g^{-1}) and in Njavara (6.66 mg g^{-1}) under OTC. The lowest total reducing sugar content was recorded in untreated plants under Open field condition i.e., 2.49 mg g^{-1} in Uma and 2.35 mg g^{-1} in Njavara. The results pertaining to total reducing sugars of leaves at grain filling stage is presented in Table11.

Table 11. Effect of zinc treatments and CO_2 concentrations on total reducing sugars of rice at grain filling stage (mg g^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	3.46	2.81	3.07	2.49	4.61	4.21	4.29	3.89	6.36	6.07	6.18	5.96	4.45
Njavara	3.37	2.71	3.03	2.35	4.79	4.27	4.49	4.11	6.66	6.46	6.49	6.25	4.58
MEAN(C)	2.9				4.3				6.3				
	CD (0.05): D=0.062, V= 0.044, C*D= 0.107, V*C=0.076, D*V= N/A												
	SE±(m): D=0.022, V=0.015, C*D=0.038, V*C=0.027, D*V=0.031												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.7 Photosynthetic rate (active tillering stage) (µmol CO_2 $m^{-2}s^{-1}$):

The influence of CO_2 levels on photosynthetic rate was highly evident in this programme. The photosynthetic rate of leaves at active tillering stage has shown significant difference among the control plants kept under different CO_2 levels without any Zinc treatment (D_4). The photosynthetic rate was was recorded highest in plants kept in OTC (27 µmol CO_2 $m^{-2}s^{-1}$) followed by those in the Polyhouse (20.9 µmol CO_2 $m^{-2}s^{-1}$) and then by the control plants (12.7 µmol CO_2 $m^{-2}s^{-1}$).

Zinc treatments did not have significantly influence on photosynthetic rate under any of the three CO_2 level. The varietal performance also did not show any significant difference in the case of photosynthetic rate under any of the CO_2 condition. The results pertaining to the photosynthetic rate of leaves at active tillering stage is presented in Table12.

Table 12. Effect of zinc treatments and CO_2 concentrations on Photosynthetic rate of rice at active tillering stage (µmol CO_2 $m^{-2}s^{-1}$):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	13.2	13	12.4	12.1	20.5	19.9	21.6	19.6	28.3	26.6	27.6	25.7	20.0
Njavara	13.1	12.9	12.6	11.9	21.8	21.4	21.7	20.5	27.9	26.8	27.5	25.7	20.3
MEAN(C)	12.7				20.9				27.0				
	CD(0.05): D=0.447,V= N/A,C*D= 0.774, V*C=0.548, D*V= N/A												
	SE±(m): D=0.157, V=0.111,C*D=0.272, V*C=0.193, D*V=0.222												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.8 Photosynthetic rate (grain filling stage) (µmol CO_2 $m^{-2}s^{-1}$):

The influence of CO_2 levels and varieties on photosynthetic rate of leaves at grain filling stage was similar to that at the active tillering stage. The photosynthetic rate was significantly different among the control plants (D4) kept under different CO_2 levels. The photosynthetic rate was recorded highest in OTC (17.6 µmol CO_2 $m^{-2}s^{-1}$) followed by Polyhouse (13 µmol CO_2 $m^{-2}s^{-1}$) and then by the control plants (9.7 µmol CO_2 $m^{-2}s^{-1}$).

The results pertaining photosynthetic rate of leaves at grain filling stage is presented in Table13.

Table 13. Effect of zinc treatments and CO_2 concentrations on Photosynthetic rate of rice at grain filling stage (µmol CO_2 $m^{-2}s^{-1}$):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	9.7	9.0	10.8	8.4	15.1	13.2	13.4	12.1	18.1	18.8	19.1	15.6	13.6
Njavara	10.8	10.6	9.6	8.6	13.8	12.9	12.7	11.0	17.6	18.0	18.3	15.6	13.3
MEAN(C)	9.7				13.0				17.6				
	CD(0.05): D=0.632,V= N/A,C*D= N/A, V*C= N/A, D*V= N/A												
	SE±(m): D=0.022, V=0.157,C*D=0.385, V*C=0.272, D*V=0.314												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.9 Transpiration rate (active tillering stage) (mmol H_2O m^{-2} s^{-1}):

The transpiration rate of leaves at active tillering stage was significantly low under elevated CO_2 levels relative to the control plants that were maintained in ambient condition.

. Highest transpiration rate was recorded in Open field condition (4.4 mmol H_2O $m^{-2}s^{-1}$) followed by Polyhouse (3.4 mmol H_2O $m^{-2}s^{-1}$) and OTC (2.4 mmol H_2O $m^{-2}s^{-1}$).

The zinc treatments or varieties did not have any significant effect on transpiration rate. The results pertaining to the transpiration rate of leaves at active tillering stage is presented in Table14.

Table 14. Effect of zinc treatments and CO_2 concentrations on transpiration rate of rice at active tillering stage (mmol H_2O m^{-2} s^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN (V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	4.49	4.36	4.37	4.16	3.13	3.17	3.29	3.21	2.42	2.24	2.51	2.44	3.32
Njavara	5.01	4.21	4.52	4.19	3.64	3.46	3.86	3.75	2.63	2.14	2.41	2.18	3.50
MEAN(C)	4.4				3.4				2.4				
	CD(0.05): D=0.142,V= 0.101,C*D= 0.247, V*C=0.174, D*V= 0.201												
	SE±(m): D=0.050, V=0.035,C*D=0.087, V*C=0.061, D*V=0.071												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.10 Transpiration rate (grain filling stage) (mmol H_2O m^{-2} s^{-1}):

Similar trend was shown in the case of photosynthetic rate under grain filling stage also. Highest transpiration rate was recorded in Open field condition (3.2 mmol H_2O $m^{-2}s^{-1}$) followed by Polyhouse (2.1 mmol H_2O $m^{-2}s^{-1}$) and OTC (1.8 mmol H_2O $m^{-2}s^{-1}$).

The Zinc treatments or varieties did not have any significant effect on transpiration rate at grain filling stage also. The results pertaining to the transpiration rate of leaves at grain filling stage is presented in Table15.

Table 15. Effect of zinc treatments and CO_2 concentrations on transpiration rate of rice at grain filling stage (mmol H_2O m^{-2} s^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	3.18	3.11	3.21	3.13	2.11	2.19	2.05	1.96	1.87	1.82	1.91	1.83	2.3
Njavara	3.26	3.14	3.28	3.12	2.18	2.07	2.13	1.94	1.86	1.76	1.74	1.83	2.36
MEAN(C)	3.2				2.1				1.8				
	CD(0.05): D=0.064,V= N/A,C*D= N/A, V*C= N/A, D*V= N/A												
	SE±(m): D=0.022, V=0.016,C*D=0.039, V*C=0.027, D*V=0.032												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.11 Stomatal conductance (active tillering stage) (mmol H_2O m^{-2} s^{-1}):

The control plants that were grown without any treatment (D4) have responded significantly to the different CO_2 levels with respect to stomatal conductance at active tillering stage. Highest stomatal conductance was recorded in open field condition (380.4 mmol H_2O $m^{-2}s^{-1}$) followed by polyhouse (304.6 mmol H_2O $m^{-2}s^{-1}$) and OTC (277.2 mmol H_2O $m^{-2}s^{-1}$).

The Zinc treatments did not have any significant effect on stomatal conductance at tillering stage. The varietal influence in this case was also not significant. The results pertaining to the stomatal conductance of leaves at active tillering stage is presented in Table16.

Table 16. Effect of zinc treatments and CO_2 concentrations on Stomatal conductance in rice at active tillering stage (mmol H_2O m^{-2} s^{-1}):

	Open field condition (C1)				Polyhouse (C2)				OTC (C3)				MEAN (V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	385	380.3	382.7	373.3	311.3	298.3	306.7	304.7	282.7	276.7	282.7	272.3	321.4
Njavara	387.3	379.7	384.3	370.3	312	302	314	287.7	282	269.7	279	272.7	320.1
MEAN (C)	380.4				304.6				277.2				
	CD(0.05): D=3.206,V= N/A,C*D= N/A, V*C= N/A, D*V= N/A												
	SE±(m): D=1.127, V=0.797,C*D=1.953, V*C=1.381, D*V=1.594												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.2.12 Stomatal conductance (grain filling stage) (mmol H_2O m^{-2} s^{-1}):

The stomatal conductance of control plants that were grown without any treatment (D_4) was significantly influenced by the different CO_2 levels with respect to stomatal conductance at grain filling stage. Highest stomatal conductance was recorded in Open field condition (305.7 mmol H_2O $m^{-2}s^{-1}$) followed by Polyhouse (287.8 mmol H_2O $m^{-2}s^{-1}$) and OTC (226 mmol H_2O $m^{-2}s^{-1}$).

The Zinc treatments did not have any significant effect on stomatal conductance at grain filling stage. The varietal influence in this case was also not significant. The results pertaining to the stomatal conductance of leaves at grain filling stage is presented in Table17.

Table 17. Effect of zinc treatments and CO_2 concentrations on Stomatal conductance in rice at grain filling stage (mmol H_2O m^{-2} s^{-1}):

	Open field condition (C1)				Polyhouse (C2)				OTC (C3)				MEAN (V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	307	302.3	306	301.3	291	285	290	286	233	218.3	232.3	216.3	272.4
Njavara	311.3	304.3	314	299.3	292.3	286.7	288.3	282.7	236.7	223	232.3	215.7	273.9
MEAN (C)	305.7				287.8				226.0				
	CD(0.05): D=3.307,V= N/A,C*D= N/A, V*C= N/A, D*V= N/A												
	SE±(m): D =1.163, V=0.822,C*D=2.014, V*C=1.424, D*V=1.644												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.3 OBSERVATIONS ON ZINC DYNAMICS OF RICE AT DIFFERENT CO_2 CONCENTARTIONS AND UNDER ZINC TREATMENTS:

4.3.1 Zinc content in leaf (μg g^{-1}):

The leaf zinc contents of control plants were found to be significantly different under different CO_2 conditions Zinc content in leaf was increasing with increasing CO_2 levels and the zinc content in leaf was highest under Open field condition (23.68 μg g^{-1}) followed by those under Polyhouse (23.31 μg g^{-1}) and OTC (23.29 μg g^{-1}).

All the treatments influenced zinc content in leaf significantly under all the three CO_2 conditions. Among the treatments, D_1 (Foliar spray) has shown highest zinc content in leaf followed by D_3, (Seed treatment) D_2 (AMF treatment) and D_4 (No treatment) under all the CO_2conditions. In the case of Uma, the highest value (25.8 μg $g^{-1)}$ was recorded by the set of plants which received 0.5% $ZnSO_4$ foliar spray under open field condition. In the case of Njavara also the same treatment was the most effective recording the highest value of 26.1 μg g^{-1} under OTC. Plants kept under OTC without any treatment (D_4) recorded lowest values, 16.7 μg g^{-1}, in the case of Uma and 17.6 μg g^{-1}, in the case of Njavara. The results pertaining to zinc content in leaf is presented in Table18.

Table 18. Effect of zinc treatments and CO_2 concentrations on Zinc content in leaf of rice (μg g^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN (V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	25.8	23.9	24.2	21.3	25.9	24.2	24.6	19.0	25.4	24.3	24.8	16.7	23.3
Njavara	24.7	23.9	24.6	21.1	25.4	24.2	24.9	18.4	26.1	25.7	25.5	17.6	23.5
MEAN(C)	23.68				23.31				23.29				
	CD(0.05): D=0.196,V= 0.139,C*D= 0.339, V*C=0.240, D*V= 0.277												
	SE±(m): D=0.069, V=0.049,C*D=0.084, V*C=0.084, D*V=0.097												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

4.3.2 Zinc content in shoot (μg g^{-1}):

The shoot Zinc content was also found to be significantly influenced by CO_2 concentration in the growing environment.

Zinc content in shoot was increasing with increasing CO_2 levels. The highest value for zinc content in shoot was recorded under Open field condition (23 μg g^{-1}) followed by those under OTC (22.8 μg g^{-1}) and then by the Polyhouse (22.6 μg g^{-1}).

All the treatments influenced zinc content in shoot significantly under all the three CO_2 conditions. Among the treatments, D_1 (Foliar spray) has shown highest zinc content in shoot followed by D_3 (seed treatment), D_2 (AMF treatment) and D_4 (No treatment) under all the conditions. The highest value recorded was 25.4 μg g^{-1} (D_1) in Uma and 26 μg g^{-1} (D_1) in Njavara under OTC. Plants kept under OTC without any treatment (D_4) recorded lowest values, 16.7μg g^{-1}, in the case of Uma and 16.6 μg g^{-1}, in the case of Njavara. The results pertaining to zinc content in shoot is presented in Table19.

Table 19. Effect of zinc treatments and CO_2 concentrations on Zinc content in shoot of rice (µg g^{-1}):

	Open field condition (C_1)				Polyhouse (C_2)				OTC (C_3)				MEAN(V)
VARIETIES (V)	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4	
Uma	24.4	22.7	23.8	20.8	24.2	24.3	24.4	18.7	25.4	24.7	24.4	16.7	22.9
Njavara	25.4	21.9	23.5	21.1	25.2	23.3	23.4	17.6	26.0	24.4	23.9	16.6	22.7
MEAN(C)	23.0				22.6				22.8				
	CD(0.05): D=0.270,V= N/A,C*D= 0.468, V*C= N/A, D*V= 0.382												
	SE±(m): D=0.095, V=0.067,C*D=0.165, V*C=0.116, D*V=0.134												

D_1-Zn Foliar spray; D_2-AMF Treatment; D_3-Seed Treatment; D_4-No Treatment

Discussion

5. DISCUSSION

Rice (*Oryza sativa* L.) is the world's most important cereal and staple food crop for more than half of the world's population. It supplies more energy in the form of calories and is a good source of thiamine, riboflavin, and niacin and is an essential source of Zn for the poor residing in rural areas (Jiang *et al*., 2008).

Zinc is an essential mineral nutrient for human beings. Zn deficiency is known to show serious impact on human health, particularly in children, such as impairments in physical growth, immune system, learning ability and causing DNA damage and cancer development (Black *et al.,* 2008).

During the last 150 years, atmospheric CO_2 concentration has increased from about 280 ppm to the current level of 400 ppm. By 2050, the earth's atmospheric CO_2 is predicted to reach about 550 ppm. Higher concentrations of CO_2 are generally known to stimulate plant photosynthesis and growth although, recent research suggests that predicting its impacts on plant growth and development is complex. However, information on the eCO_2 effects on nutritional quality in relation to major nutrients and therefore the health-promoting phytochemicals in food crops indicate that major nutrients in food crops including protein, phosphorus, potassium, calcium, iron, zinc and other micronutrients in many food crops are reduced at eCO_2 levels (Rajashekar, 2018). This was also reported in the case of wheat, barley and rice grown in controlled-environment chambers (Myers *et al*., 2014).

The present programme "Influence of elevated CO_2 on zinc dynamics in rice (*Oryza sativa* L.)" was formulated with the objective of assessing the impact of elevated CO_2 on growth, development and nutritional quality of rice in relation to modifications in Zn dynamics.

In this study, two rice genotypes, Njavara-golden yellow collected from RARS, Pattambi and Uma [MO16]) collected from IFRI, Karamana were exposed to different CO_2 levels by raising them under different conditions i.e., OTC system with 500 ppm CO_2, Polyhouse and Open field condition. They were also subjected to different Zn enrichment treatments. The experimental plants were evaluated for growth, physiological and biochemical parameters and parameters related to zinc dynamics. Significant variations were observed for all the parameters studied and the results obtained are discussed in this chapter with appropriate support from previous studies.

5.1 EFFECT OF ELEVATED CO_2 ON GROWTH PARAMETERS:

In this study, various growth parameters such as plant height (cm), days to 50% flowering, no. of tillers, root weight (g/plant), and straw yield (g/plant) as influenced by different CO_2 levels were studied in the rice genotypes Uma and Njavara.

In the case of plant height, the control plants which were maintained without any treatment (D4) were found to be responding significantly to the different CO_2 levels. Plant height was increasing with increasing CO_2 concentration and the highest mean plant height was recorded under OTC condition (108cm) followed by those under Polyhouse (97cm) and control plants (89cm).

A study was conducted by Liu *et al*. (2017) in rice using a simulation experiment and climatic chambers, with the rice variety Liangyou 287 with eCO_2 concentration of 550 μmol mol $^{-1}$ and ambient CO_2 concentration of 400 μmol mol $^{-1}$. In this study, as the rice growing stage prolonged and as CO_2 increased plant height which is an important trait closely related to rice yield, showed an increase of 19.94%.

The result of present study is in accordance with the above research work. In this study there was an increase in plant height by 21.35% under OTC and 9% under Polyhouse compared to Open field condition. It follows that altered plant structure induced by exposure to eCO_2 might be due to the result of higher rates of cell division, increased cell expansion and altered patterns of primordium initiation (Pritchard *et al*., 1999). It is possible that growth stimulation of plants grown under eCO_2 may be direct (based on substrate supply) or indirect (based on chemical signals) or both Increased photosynthate availability in meristems increases the proportion of rapidly dividing cells by stimulating cyclin activity (regulatory subunits of a family of protein kinases, which facilitates the transition of cells from G0 to G1 of the cell cycle), thus stimulating division (Jacobs, 1997). Among the treatments, D_1

(Foliar spray) has shown highest plant height followed by D_2 (AMF treatment), D_3 (seed treatment) and D_4 (No treatment) in all three CO_2 conditions. Zn is entangled in cell elongation and/or increased cell division rates (Cakmak, 2000; Ramesh *et al.*, 2014), and meristematic growth (Abaid-Ullah *et al.*, 2015) which ultimately led to increase in plant height compared to untreated plants. There was an increase of 24.58%, 19.44% and 15.69% in the case of Uma and 48.47%, 13.83% and 3.35% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment) and in D_3 (seed treatment) respectively in plant height under open field compared to D_4 (without treatment). There was an increase of 24.27%, 16.22% and 8.17% in the case of Uma and 40%, 18.14% and 6.7% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment) and in D_3 (seed treatment) respectively in plant height under polyhouse compared to D_4 (without treatment). There was an increase of 15.5%, 13.48% and 8.38% in the case of Uma and 19.33%, 17.99%, 11.96% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3(seed treatment) respectively in plant height under OTC compared to D_4 (without treatment).(Table1.) (Fig.1).

The number of tillers has shown significant difference among the control plants kept under different CO_2 levels that were grown without any treatment (D_4). Highest no. of tillers was recorded in OTC (30.1) followed by the plants kept in the Polyhouse (21.1) and control plants (18).

According to Yang *et al.* (2006), a study in rice using FACE facility (200 μmol mol^{-1} above ambient CO_2 concentration) improved the maximum tiller number m^{-2} (+30.3%). The result of present experiment is also similar to the above research which has enhanced the tiller number under eCO_2 by 58.3% under OTC and 8.3% under Polyhouse compared to Open field condition. Senewera *et al.* (2003) claimed that plants grown under eCO_2 produced more ethylene than the plants grown under ambient CO_2. Increase in ethylene production is one of the key features of accelerated growth and development in rice under eCO_2 that improves no. of tillers and development of auxiliary bud.

An experiment was carried out in OTC on ginger at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India which was enriched with 500 ppmCO_2. The maximum numbers of tillers were found in the variety. Aswathy under eCO_2 when compared to ambient CO_2 (Manasa *et al.*, 2020).

Among the treatments, D_1 (Foliar spray) has shown highest no. of tillers followed by D_2 (AMF treatment), D_3 (seed treatment) and D_4 (No treatment) in all three CO_2 conditions. Chang *et al.* (2005) also demonstrated that Zn is essential for cell division and elongation as it is essential for the fundamental events of growth. Superior enzymatic activity and auxin metabolism might have improved tiller number of rice with Zn supply in the present study. There was an increase of 37.3%, 30% and 36% in the case of Uma and 71.9%, 36% and 30% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment) and in D_3 (seed treatment) respectively in no. of tillers under open field compared to D_4 (without treatment). There was an increase of 21.2%, 4.6% and 6% in the case of Uma and 24%, 7.8% and 19.8% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment) and in D_3 (seed treatment) respectively in no. of tillers under polyhouse compared to D_4 (without treatment). There was an increase of 18.5%, 7% and 11.5% in the case of Uma and 30%, 18.6%, 20.2% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3(seed treatment) respectively in no. of tillers under OTC compared to D_4 (without treatment) (Table3.) (Fig.3).

The number of days to 50% flowering has shown significant difference to different CO_2 levels when compared to the control plants without any treatment (D_4). The shortest number of days to 50% flowering was recorded in Open field condition (67.8) followed by those Polyhouse (75.5) and OTC (78.3).

Shimono *et al.* (2008) reported that under FACE facility eCO_2 (575 ± 35 mmol mol^{-1}) delayed flowering date of all rice cultivars (Kirara 397, Kakenashi and Akitkomachi) by 5-6 days compared to ambient CO_2 (385 ± 14 mmol mol^{-1}). The present study revealed that the no. of days to 50% flowering was delayed by 10-11 days under OTC and Polyhouse relative to Open field condition. The Zn treatments did not result in any significant difference in the time taken to reach 50% flowering.

(Table2.) (Fig.2). C_3 plants grown under eCO_2 commonly accumulate excess carbohydrates in leaves (Curtis and Wang, 1998; Long *et al*., 2004), which can have an influence on flowering time. Several mutants of *Arabidopsis thaliana* such as cam1, pgm (AT5G51820), adg1 (AT5G48300) and sex1 (AT1G10760), which exhibited altered starch metabolism and enhanced tissue sugar concentrations, also exhibited late-flowering (Caspar *et al*., 1985; Lin *et al*., 1988; Eimert *et al*., 1995).

In the case of root weight, the control plants which were maintained without any treatment (D_4) responded significantly to the different CO_2 levels. Root weight was increasing with increasing CO_2 levels and the highest root weight was recorded under OTC condition (19.1g/plant followed by those under Polyhouse (16.8g/plant) and then by the control plants (14.2g/plant). The straw yield was significantly influenced by different CO_2 levels. Highest straw yield was recorded by the experimental plants kept in OTC (36.6g/plant) followed by those in Polyhouse (27.5g/plant) and then by the control plants (22.2g/plant).

Rice (IR-36) grown in phytotron with approximately 31°C day/ 23°C night temperature, eCO_2 concentration of 660 μmol mol^{-1}recorded 37% enhancement in root weight (g/plant) and 18% enhancement in shoot weight (g/plant) compared to ambient CO_2 (360 μmol mol^{-1}) (Ziska and Teramura, 1992).

A pot culture experiment was conducted with three varieties of amaranthus i.e, Arun, CO-1 and Renusree at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The technology used for CO_2 enrichment was Open Top Chamber (OTC) system. Two Open Top Chambers were used, one with CO_2 level of 600 ppm and a second control chamber with control chamber level for assessing chamber effect. A set of experimental plants was maintained in the open field as control. Significantly higher root weight was observed under elevated CO2 (0.92 g) compared to control chamber (0.69g) and open control (0.53 g) (Chatti and Manju, 2018).

The result of the present study was also similar to the above studies which have increased the root weight (g/plant) by 34.5% under OTC and 18.31% under Polyhouse compared to Open field condition. Salsman *et al*. (1999) reported that the rise in root weight under eCO_2 is linked with the improved starch levels in roots and an increment in the ABA levels that might have caused more carbon to get accumulated for root growth. Plant growth is induced by both above ground and below-ground processes under eCO_2. Below ground processes of plants accelerates photosynthesis through nutrient and water uptake, which then induce the above ground biomass production (Madhu and Hatfield, 2013).

Among the treatments, D_2 (AMF treatment) has shown highest root weight. AMF colonize roots and can develop the adaptability of host plants by offering additional phosphorus, nitrogen and zinc to plants. Root systems were extended, enhancing the root surface that is utilized for nutrient uptake by more than 100-fold, by symbiosis with AMF in an experiment conducted in upland cotton (*Gossypium hirsutum* L.) (Gao *et al*., 2020). Hippler *et al.* (2015) reported that nearly 2800 proteins need Zn for their structural and functional integrity and hence there is a high necessity for Zn during root and coleoptiles development for active protein synthesis. AMF treatment had maximum influence on root weight in the cases of both the varieties with 101.83% and 106.76% increase when compared to control in the case of Uma and Njavara respectively. AMF treatment was followed by seed treatment and foliar spray in influencing root weight. Though the extent of influence differed, the trend was similar under ambient condition, polyhouse and in OTC system in both the varieties. The straw yield was significantly influenced by different CO_2 levels. Highest straw yield was recorded by the experimental plants kept in OTC (36.6g/plant) followed by those in Polyhouse (27.5g/plant) and then by the control plants (22.2g/plant).

The straw yield (g/plant) was enhanced under eCO_2 by 64.9% under OTC and 23.9% under Polyhouse compared to Open field condition. Response of plant growth to eCO_2 is also associated with the source-sink relationship of the plant (Makino and Mae, 1999). Elevated CO_2 increases the carbon source activity that results in an increased rate of photosynthetic CO_2 assimilation providing more carbohydrates (Paul and Foyer, 2001) which are used by plants to advance supplementary sinks such as new tillers and secondary shoots leading to enhanced straw yield. The developmental plasticity of these organs determines the final growth response to eCO_2.

A study was conducted by Srikanth (2019) on cowpea variety Lola at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala under OTC with 600 ppm eCO_2 concentration. In this xperiment highest value for shoot weight was observed in variety Lola (65.05 g) under eCO_2 compared to ambient CO_2 condition.

All the zinc enrichment treatments influenced straw yield significantly under all the three CO_2 conditions. Among the treatments, D_1 (Foliar spray) has shown highest shoot weight. There was an increase of 32.6%, 7.4% and 16% in the case of Uma and 19.2%, 1.31% and 6.6% in the case of Njavara in D_4 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in shoot weight under open field compared to D_4 (without treatment). There was an increase of 38.1%, 14.9% and 24.1% in the case of Uma and 27.5%, 13.3% and 20.4% in the case of Njavara in D_1, D_2 and D_3 respectively in shoot weight under polyhouse compared to D4 There was an increase of 28.2%, 2% and 6.2% in the case of Uma and 23.2%, 14.5%, 16.6% in the case of Njavara in D_1, D_2 and D_3 respectively in shoot weight under OTC compared to D_4 (without treatment) (Table5.) (Fig.5).

Krishnan *et al.* (2007) has utilized two popular models of rice growth ORYZA1 and INFOCROP rice are used in a study. Prior to their use, both were evaluated and compared and then these crop models were calibrated for the Indica variety IR 36. The rice-growing regions included for the present study lie in eastern India, and these sites (Bhubaneswar, Chinsurah, Cuttack, Faizabad, Jabalpur, Jorhat, Kalyani, Pusa, Raipur and Ranchi). At the CO_2 levels tested under OTC (700 ppm), both the models predicted the declining yields of rice due to an increase in temperature. At the ambient CO_2 level (considered at 380 ppm), ORYZA predicted a mean change of -7.20% in yields for every 1°C increase in temperature, while INFOCROP predicted -6.66%. But increasing CO_2 concentration (700 ppm) resulted in the increase of 30.37 and 56.37% in yield by ORYZA and INFOCROP, respectively without any increment in temperature. However, with temperature increase of +4°C above ambient level, the differences in the yield predictions by the two models were -23.59% and -17.35% respectively.

A FACE experiment by Wang *et al.* (2018) was conducted in high-yield japonica rice (NJ9108) crop was grown under eCO_2 (200 µmol mol^{-1} above the canopy of the ambient plot) where the daily mean air temperature was 33°C and for ambient plot the daily mean air temperatue was 29°C. As the daily mean air temperature exceeded 33°C during the panicle initiation stage there was a decrease in grain yield (1100.3 gm^{-2}) relative to the grain yield of ambient plot (1145.2 gm^{-2}).

In the present study, the observations were in coincidence with above studies. As the temperature has exceeded beyond the daily mean air temperature during grain filling stage there was chaffiness in the panicles of OTC because of which there was no possibility of recording the yield parameters and quality parameters of grain in OTC.

5.2 EFFECT OF ELEVATED CO_2 ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS:

In this study, various physiological and biochemical parameters such as total chlorophyll content, total soluble protein content, total reducing sugars content, photosynthetic rate, transpiration rate, stomatal conductance were studied in the two rice genotypes and this section explains the basis of results obtained along with similar studies.

The total chlorophyll content of leaves in active tillering stage was significantly high under elevated CO_2 levels relative to the control plants that were maintained in ambient condition. Highest chlorophyll content was recorded in plants under Polyhouse condition (2.33 mg g^{-1} fresh weight) followed by those under OTC (2.18 mg g^{-1} fresh weight) and control plants (2.06 mg g^{-1} fresh weight). Similarly, the total chlorophyll content of leaves in grain filling stage has also shown significant difference at different CO_2 levels by the control plants. Highest chlorophyll content was observed in plants kept in OTC (1.805 mg g^{-1} fresh weight) followed by those in the Polyhouse (1.803 mg g^{-1} fresh weight) and then by the control plants (1.684 mg g^{-1} fresh weight).

The photosynthetic rate of leaves at active tillering stage has shown significant difference among the control plants kept under different CO_2 levels without any Zinc treatment (D_4). The highest photosynthetic rate was recorded in plants kept in OTC (27 µmol CO_2 $m^{-2}s^{-1}$) followed by those in the Polyhouse (20.9 µmol CO_2 $m^{-2}s^{-1}$) and then by the control plants (12.7 µmol CO_2 $m^{-2}s^{-1}$). The influence of CO_2 levels and varieties on photosynthetic rate of leaves at grain filling stage was similar to that at the active tillering stage. The highest photosynthetic rate was recorded in OTC (17.6 µmol CO_2 $m^{-2}s^{-1}$) followed by Polyhouse (13 µmol CO_2 $m^{-2}s^{-1}$) and then by the control plants (9.7 µmol CO_2 $m^{-2}s^{-1}$).

In the case of stomatal conductance, the control plants that were grown without any treatment (D_4) have responded significantly to the different CO_2 levels with respect to stomatal conductance at active tillering stage. Highest stomatal conductance was recorded in open field condition (380.4 mmol H_2O $m^{-2}s^{-1}$) followed by polyhouse (304.6 mmol H_2O $m^{-2}s^{-1}$) and OTC (277.2 mmol H_2O $m^{-2}s^{-1}$). The influence of CO_2 levels and varieties on stomatal conductance of leaves at grain filling stage was similar to that at the active tillering stage. Highest stomatal conductance was recorded in open field condition (380.4 mmol H_2O $m^{-2}s^{-1}$) followed by polyhouse (304.6 mmol H_2O $m^{-2}s^{-1}$) and OTC (277.2 mmol H_2O $m^{-2}s^{-1}$).

Wang *et al.* (2020a) conducted a CO_2 enrichment experiment in rice. Early rice cv. Liangyou 287 and late rice cv. Xiangfengyou 9 were grown in OTC (60 µmol mol^{-1} above ambient concentration). Elevated CO_2 stimulated photosynthetic rate at tillering and heading satge in both early rice and late rice cultivars, showing an average increase of 9.4 % in photosynthetic rate compared with ambient CO_2. At maturity, photosynthetic rate decreased by 8.4 % under the eCO_2 treatment, indicating that the stimulation effect on leaf photosynthesis induced by eCO_2 completely vanished by maturity. Elevated CO_2 reduced stomatal conductance (g_s) by 9.0 % in both early rice and late rice cultivars compared with ambient CO_2. Elevated CO_2 tended to increase leaf chlorophyll content at pre-heading stages. At post-heading stages, eCO_2 showed no significant impact at milking stage but decreased at maturity. Even under eCO_2 condition, there was a reduction in cholrophyll content at maturity, as shown by a decrease of 9.8 % compared with ambient CO_2 concentration.

An experiment was done on the reproductive physiology of tomato variety Vellayani Vijay using OTC at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala with eCO_2 concentration of 500 ppm. Higher values for total chlorophyll content (1.74 mg g^{-1}) was observed relative to ambient CO_2 condition (Ajay, 2019).

The results of the present study are in agreement with Wang *et al.* (2020a). In this study, there was an enhancement in the total chlorophyll content under eCO_2 by 6.3% under OTC and 13.7% under Polyhouse compared to Open field condition. Increasing levels of CO_2 can lead to increased efficiency of PS II and also reduced risk of damage caused to PSII by oxidative stress and a better chlorophyll status (Wang *et al.*, 2015).

Among the treatments, D_1 (Foliar spray) has shown highest total chlorophyll content. Zinc being a structural and catalytic component of proteins and enzymes and also as a co-factor plays a very vital role in pigment biosynthesis (Balashouri, 1995). There was an increase of 6.9%, 6.1% and 5.5% in the case of Uma and 4.4%, 34.6% and 12.7% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total chlorophyll content under open field compared to D_4 (without treatment). There was an increase of 5.4%, 4.03% and 2.01% in the case of Uma and 31%, 8.6% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total chlorophyll content under polyhouse compared to D_4 (without treatment). There was an increase of 4.03%, 0.7% and 0.7% in the case of Uma and 20%, 2.3%, 17% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3(seed treatment) respectively in total chlorophyll content under OTC compared to D_4 (without treatment) at active tillering stage (Table6) (Fig.6).

The total chlorophyll content was increased at grain filling stage under eCO_2 by 7.19% under OTC and 7.07% under Polyhouse compared to Open field condition. Among the treatments, D_1 (Foliar spray) has shown highest total chlorophyll content. There was an increase of 2.9%, 1.4% and 2.14% in the case of Uma and 2.7%, 15.1% and 8.11% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total chlorophyll content under open field compared to D_4 (without treatment). There was an increase of 6.3%, 6.3% and 4.23% in the case of Uma and 4%, 11.4% and 8% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total chlorophyll content under polyhouse compared to D_4 (without treatment). There was an increase of 2.7%, 2.03% and 1.4% in the case of Uma and 8.7%, 6.2%, 2% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3(seed treatment) respectively in total chlorophyll content under OTC compared to D_4(without treatment). However, the total chlorophyll content was lowered at grain filling stage than at active tillering stage. This might be because of senescence in leaves at later stages (Melati *et al.*, 2016) (Table.7) (Fig.7). Responses of plants to eCO_2 are much greater at early vegetative stages when compared to later stages Senewera *et al.*, 2003).

The photosynthetic rate in the present study also revealed that there was an enhancement under eCO_2 by 112.6% under OTC and 64.6% under Polyhouse compared to Open field condition at active tillering stage and at grain filling stage there was an increase of 81.4% under OTC and 34.02% under Polyhouse relative to open field condition. The stimulation of photosynthesis was obvious under eCO_2 at early growth stages, such as tillering stage. CO_2 is a substrate for photosynthesis and so its increased availability can increase the velocity of carboxylation and simultaneously suppresses photorespiration (Long, 1991; Drake *et al.*, 1997). Influence of Zn treatments on photosynthetic rate was not significant and varieties did not vary significantly to different CO_2 concentrations. However, the photosynthetic rate was lowered at grain filling stage than at active tillering stage (Table.12&13) (Fig.12&13). Leaf senescence and low chlorophyll content could explain the disappearance of CO_2 stimulation at later stages of rice plant.

Stomatal conductance was decreased under eCO_2 by 27.13% under OTC and 19.9% under Polyhouse compared to open field condition at active tillering stage and at grain filling stage there was an increase of 26.07% under OTC and 5.9% under Polyhouse relative to open field condition. Responses to Zn treatment and varietal performances did not vary significantly under different CO_2 levels (Table.16&17)

(Fig.16&17). Elevated CO_2 induced partial closure of stomatal closure (Shimono *et al.*, 2010; Cai *et al.*, 2018) which might have led to decreased stomatal conductance in this study.

The transpiration rate of leaves at active tillering and grain filling stage was significantly low under elevated CO_2 levels relative to the control plants that were maintained in ambient condition. Highest transpiration rate at active tillering stage was recorded in Open field condition (4.4 mmol H_2O $m^{-2}s^{-1}$) followed by Polyhouse (3.4 mmol H_2O $m^{-2}s^{-1}$) and OTC (2.4 mmol H_2O $m^{-2}s^{-1}$). Highest transpiration rate at grain filling stage was recorded in Open field condition (3.2 mmol H_2O $m^{-2}s^{-1}$) followed by Polyhouse (2.1 mmol H_2O $m^{-2}s^{-1}$) and OTC (1.8 mmol H_2O $m^{-2}s^{-1}$).

A study was conducted by Imai and Okamoto-Sato (1991) in rice (*Oryza sativa* L. cv. Nipponbare) under eCO_2 with CO_2 controllers (Fuji model ZEP-6). The ambient CO_2 concentration was 350 µmol mol^{-1} and eCO_2 concentration was 500 µmol mol^{-1} . The transpiration rate was decreased under eCO_2 (1.8) relative to ambient CO_2 condition (1.9) (Table.14&15) (Fig.14&15).

An experiment was conducted under eCO_2 in OTC (500 ppm) on black pepper varieties viz. Panniyur 1, Panniyur 5 and Karimunda at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The transpiration rate was decreased under eCO_2 (0.72 mmol H_2O m^{-2} s^{-1}) in all varieties compared to ambient CO_2 condition (Minu *et al.*, 2015).

The present study has also shown decrease in transpiration rate under eCO_2 by 45.5% under OTC and 22.7% under Polyhouse compared to open field condition at active tillering stage and at grain filling stage there was an increase of 43.8% under OTC and 34.4% under Polyhouse relative to open field condition. Responses to Zn treatment and varietal performances did not vary significantly under different CO_2 levels (Table.10&11) (Fig.10&11). In the present experiment, the direct cause for the decrease of transpiration rate was ascribed to the sensitive stomatal closure in the plants (Akitha and Tanaka, 1973). Elevated CO_2 levels have also been mentioned as the ideal antitranspirant (Carlson *et al.*, 1979). Similar results were reported by other researchers (Credyt *et al.*, 2019; Pan *et al.*, 2020; Padhy *et al.*, 2020).

In the case of total soluble protein, there was significant difference among the control plants which were maintained without any treatment (D_4) under different CO_2 levels in terms of total soluble protein at active tillering stage. Highest total soluble protein was recorded in Open field condition (3.1 mg g^{-1}) followed by polyhouse (1.1 mg g^{-1}) and OTC (0.8 mg g^{-1}) systems. Similar trend was observed at grain filling stage also. Highest total soluble protein at grain filling stage was recorded in Open field condition (7.1 mg g^{-1}) followed by Polyhouse (5.9 mg g^{-1}) and OTC (4.0 mg g^{-1}).

A study was conducted in rice variety CO 51 in different conditions i.e., ambient CO_2condition (400±9 ppm), OTC+ ambient CO_2 condition (400±9 ppm) and OTC+ eCO_2 (550 ppm). The increase in total soluble protein content was observed from tillering stage to the heading stage and a gradual decrease towards the grain filling stage. The highest soluble protein content (5.07, 8.03 and 7.11 mg g^{-1}) was observed in ambient condition at 30, 60 and 85 DAT followed by OTC + ambient CO_2 (4.95, 7.29 and 6.74 mg g^{-1}) and the lowest was registered in OTC + elevated CO_2 (4.52, 6.98 and 6.24 mg g^{-1}) (Vinothini *et al.*, 2019).

The present study was also showing similar result in total soluble protein content in leaves at active tillering stage which was decreased under eCO_2 by 74.2% under OTC and 64.5% under Polyhouse compared to Open field condition. Exposure of plants to eCO_2 results in acclimation of photosynthesis with down-guideline of the degree of Rubisco protein. This coarse control of the degree of Rubisco protein serves to improve CO_2 acquiring with use of the fixed carbon. Notwithstanding coarse control of Rubisco protein, there are fine controls, which react quickly to changes in the atmosphere. In such manner, two important components are known to manage Rubisco action. One includes the reversible carboxylation of lysine buildup within the dynamic site to actuate the enzyme, while the different operates by the reversible binding of 2-carboxyarabinitol-1 phosphate, the Rubisco dark inhibitor, to the carboxylate site (Zhu *et al.*, 2018). This diminishing total soluble protein content may reflect a general decrease of leaf protein due to reallocation of nitrogen inside the plants.

Among the treatments, D_1 (Foliar spray) has shown highest total soluble protein content. Zn is a constituent of RNA polymerase enzyme associated with N metabolism. Low Zn levels lowers activity of RNA polymerase leading to the loss of structural integrity of ribosomes and degradation of RNA. This lowered activity of RNA polymerase inhibits protein synthesis leading to accumulation of amino acid (Gonzalez *et al.*, 2018). Zn is needed as structural and catalytic constituent of protein and enzymes for standard growth and development (Broadley *et al.*, 2007) which might have led to enhanced protein content with zinc supply. There was an increase of 4%, 1.3% and 3% in the case of Uma and 3.6%, 1.9% and 2.9% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total soluble protein content under open field compared to D_4 (without treatment). There was an increase of 35.4%, 24% and 16.7% in the case of Uma and 10%, 0.91% and 1.9% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total soluble protein content under polyhouse compared to D_4 (without treatment). There was an increase of 32.3%, 10.8% and 23.08% in the case of Uma and 21.05%, 6.6%, 18.4% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3(seed treatment) respectively in total soluble protein content under OTC compared to D_4 (without treatment) (Table.8) (Fig.8).

The total soluble protein content at grain filling stage was also decreased under eCO_2 by 43.66% under OTC and 16.9% under Polyhouse compared to Open field condition. Among the treatments, D_1 (Foliar spray) has shown highest total soluble protein content. There was an increase of 0.4%, 0.14% and 0.85% in the case of Uma and 1.3%, 1.9% and 1.3% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total soluble protein content under open field compared to D_4 (without treatment). There was an increase of 4.1%, 6% and 0.3% in the case of Uma and 2.7%, 4.2% and 1.7% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total soluble protein content under polyhouse compared to D_4 (without treatment). There was an increase of 10.08%, 6.5% and 9.8% in the case of Uma and 13.5%, 3.17%, 5.8% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3(seed treatment) respectively in total soluble protein content under OTC compared to D_4 (without treatment) (Table.9) (Fig.9).

The total reducing sugars of leaves at active tillering stage was significantly high under elevated CO_2 levels relative to the control plants that were maintained in ambient condition. The highest total reducing sugar content was recorded in OTC (1.9 mg g^{-1}) followed by Polyhouse (1.3 mg g^{-1}) and then by the control plants (1.2 mg g^{-1}). The total reducing sugars of leaves at grain filling stage was also significantly high under elevated CO_2 levels relative to the control plants that were maintained in ambient condition. The highest total reducing sugar content was observed in OTC (6.3 mg g^{-1}) followed by those in Polyhouse (4.3 mg g^{-1}) and then by the control plants (2.9 mg g^{-1}).

A study conducted by Aranjuelo *et al.* (2015), revealed that increased photosynthesis rate under eCO_2 resulted in more production of sugars including glucose, fructose and raffinose across a range of crop plants.

The present study also was showing similar trend in total reducing sugars content in leaves at active tillering stage which was increased under eCO_2 by 58.3% under OTC and 8.3% under Polyhouse compared to Open field condition. Increased levels of CO_2 increases photosynthetic rate which is accompanied with the rise in sink capacity that is associated with the accumulation of higher no. of end products of photosynthesis within in the leaves. Higher sink plasticity and sink potential in the genotypes were associated with higher sugar synthesis in plants (McKinley *et al.*, 2018). It can even be expected that at eCO_2, plant shoot apex activity in terms of cell division rates and branching could increase at the tillering stage. This might cause enhanced photosynthesis leading to production of higher amounts of carbohydrates compared to ambient conditions (Jitla *et al.*, 1997).

Among the treatments, D_1 (Foliar spray) has shown highest total reducing sugars content. Zn is a cofactor of carbonic anhydrase enzyme. Carbonic anhydrase functions in fixation of photosynthetic CO_2 by elevating CO_2 concentration in chloroplast, catalysing conversion of CO_2 and water into bicarbonates and helping in transport and distribution of CO_2 through plasma membrane and chloroplast

(Gonzalez *et al*., 2018). There was an increase of 13.3%, 3.5% and 4.4% in the case of Uma and 10.9%, 4.6% and 7.2% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total reducing sugar content under open field compared to D_4 (without treatment). There was an increase of 25.6%, 26.2% and 24.4% in the case of Uma and 32.2%, 3.5% and 6.09% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total reducing sugar contents under polyhouse compared to D_4 (without treatment). There was an increase of 27.6%, 3.8% and 8.7% in the case of Uma and 36.4%, 23.03%, 1.21% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total reducing sugar contents under OTC compared to D_4 (without treatment) (Table.8) (Fig.8).

The total reducing sugars content at grain filling stage was also showing similar trend under eCO_2. It was increased by 117.24% under OTC and 48.28% under Polyhouse compared to Open field condition. Among the treatments, D_1 (Foliar spray) has shown highest total reducing sugars content. There was an increase of 39%, 12.9% and 23.3% in the case of Uma and 43.4%, 15.3% and 29% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total reducing sugars content under open field compared to D_4 (without treatment). There was an increase of 18.5%, 8.2% and 10.28% in the case of Uma and 16.6%, 3.9% and 9.3% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in total reducing sugars content under Polyhouse compared to D_4 (without treatment). There was an increase of 6.7%, 1.9% and 3.7% in the case of Uma and 6.6%, 3.4%, 3.9% in the case of Njavara in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3(seed treatment) respectively in total reducing sugar content under OTC compared to D_4 (without treatment) (Table.9) (Fig.9).

5.3 EFFECT OF ELEVATED CO_2 ON ZINC DYNAMICS IN RICE:

In this study, parameters related to zinc dynamics such as zinc content in leaf and shoot were analyzed.

Zinc is an essential trace mineral that people need to stay healthy. It is present in the cells throughout the body which is required for the defensive system of the body to properly work that plays a vital role in cell division, cell growth, wound healing, and the breakdown of carbohydrates. During pregnancy, infancy and childhood the zinc is crucial for the body to grow and develop properly.

Global CO_2 emissions continue to rise recording 405 ppm atmospheric CO_2 in 2017 (Dlugokencky and Tans, 2018). One of the consequential impacts of increasing CO_2 concentration is on food security (Field *et al*., 2014). Since CO_2 is a substrate for photosynthesis, its increasing concentration in the atmosphere can enhance accumulation of carbohydrates in plants leading to increase in crop productivity but several studies reported reduction in grain quality of cereals and pulses affecting human health (Myers *et al*., 2014).

Since CO_2 is a substrate for photosynthesis, its increasing concentration in the atmosphere can enhance accumulation of carbohydrates in plants which can lead to "dilution effect" resulting in decreased content of Zn (Ujjie *et al* ., 2019).

Zinc transfers from roots to shoots, predominantly with the transpiration stream Wu *et al*., 2010). Zn accumulation in economic parts of plant is not only related to root uptake but also depends on internal redistribution and remobilization of stored Zn within the plants (Palmgren *et al*., 2008). The only vascular tissue to reach the developing grain is the phloem (Patrick *et al*., 2001). In the developing seed, nutrients have to quit the xylem at some stage during long-distance transport and become actively loaded into the phloem. Increase in CO_2 lower the expression of transporter genes like Os ZIP5, Os ZIP3, Zn transporter precursor reducing retranslocation to panicle at grain-filling stage (Ujjie *et al*., 2019).

The results of the present study give strong evidence for the influence of CO_2 levels on Zinc status of plant tissues. Here, zinc contents of plants were found to be significantly different under different CO_2 conditions. Zinc content in leaf was decreasing with increasing CO_2 levels and the zinc content in leaf was highest under Open field condition (23.68 µg g^{-1}) followed by those under Polyhouse (23.31 µg g^{-1}) and OTC (23.29 µg g^{-1}). The shoot Zinc content was also found to be significantly influenced by CO_2 concentration in the growing environment. The highest value for zinc content in shoot was recorded under Open field condition (23 µg g^{-1}) followed by those under OTC (22.8 µg g^{-1}) and then by the Polyhouse (22.6 µg g^{-1}).

A study was conducted by Wang *et al.* (2020b) in rice crop (*Oryza sativa* L. cv., Changyou No.5) in FACE facility with eCO_2 of 500 ppm and ambient CO_2 of 410 ppm. In this study the grain Zn content decreased by 5.8% under eCO_2 relative to ambient CO_2 condition.

In the present study, the result of zinc content analyzed in leaf and stem is in agreement with the previous study which has shown decrement under eCO_2. When the plants were treated with zinc supplements the zinc content was increased compared to untreated plants under eCO_2.

Among the treatments, D_1 (Foliar spray) has shown higher zinc content in leaf followed by D_3 (seed treated), D_2 (AMF treated) and D_4 (No treatment) in the respective conditions. There was an increase of 21.1%, 12.2%, 13.6% (Uma) and17.1%, 13.2%, 16.6% (Njavara) in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in zinc content of leaf under open field compared to D_4 (without treatment). There was an increase of 36.6%, 27.4%, 29.5% (Uma) and 38%, 31.5%, 35.3% (Njavara) in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in zinc content of leaf under polyhouse compared to D_4 (without treatment). There was an increase of 52%, 45.5%, 48.5% (Uma) and 48.3%, 46%, 44.9% (Njavara) in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in zinc content of leaf under OTC compared to D_4 (without treatment) (Table.18) (Fig.18).

Similarly, in rice shoot also D_1 (Foliar spray) has shown higher zinc content followed by D_3 (seed treated), D_2 (AMF treated) and D_4 (No treatment) in the respective conditions. There was an increase of 17.3%, 9.1%, 14.4% (Uma) and 20.4%, 3.8%, 11.4% (Njavara) in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in zinc content of stem under open field compared to D_4 (without treatment).There was an increase of 29.4%, 29.94%, 30.5% (Uma) and 43.1%, 32.3%, 32.9% (Njavara) in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in zinc content of stem under polyhouse compared to D_4 (without treatment). There was an increase of 52%, 47.9%, 46.1% (Uma) and 56.6%, 46.9%, 44% (Njavara) in D_1 (Zn foliar spray treatment), in D_2 (AMF treatment), in D_3 (seed treatment) respectively in zinc content of stem under OTC compared to D_4 (without treatment) (Table.19) (Fig.19).

The result of present study indicated that zinc dynamics was improved with the zinc enrichment techniques. Foliar spray with 0.5% $ZnSO_4$ was found to be the most effective treatment in improving Zinc status in rice varieties Uma and Njavara. Foliar applied Zn is easily taken up and transferred through phloem as per the research conducted in wheat variety Aroona to test the extent of Zn applied foliarly for their suitability to render sufficient Zn nutrition in the food supply (Haslett *et al.*, 2001).

Figure1. Effect of elevated CO_2 and zinc treatments on plant height (cm) of rice:

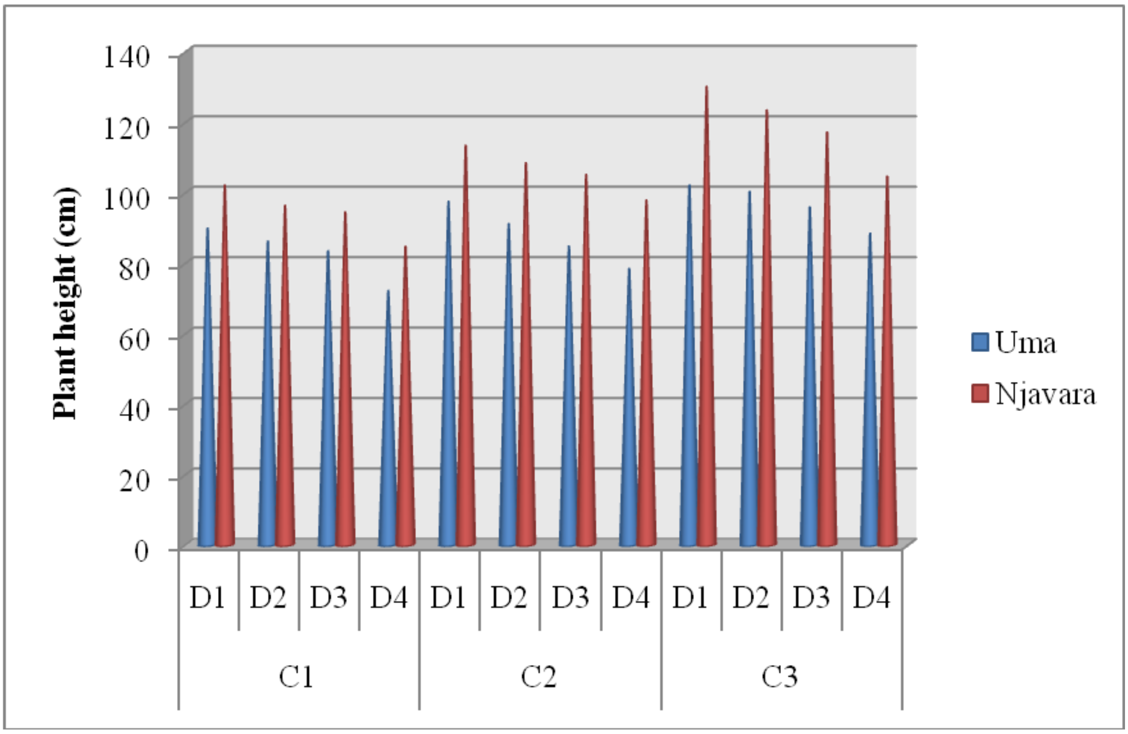


Figure2. Effect of elevated CO_2 and zinc treatments on days to 50% flowering ofrice:

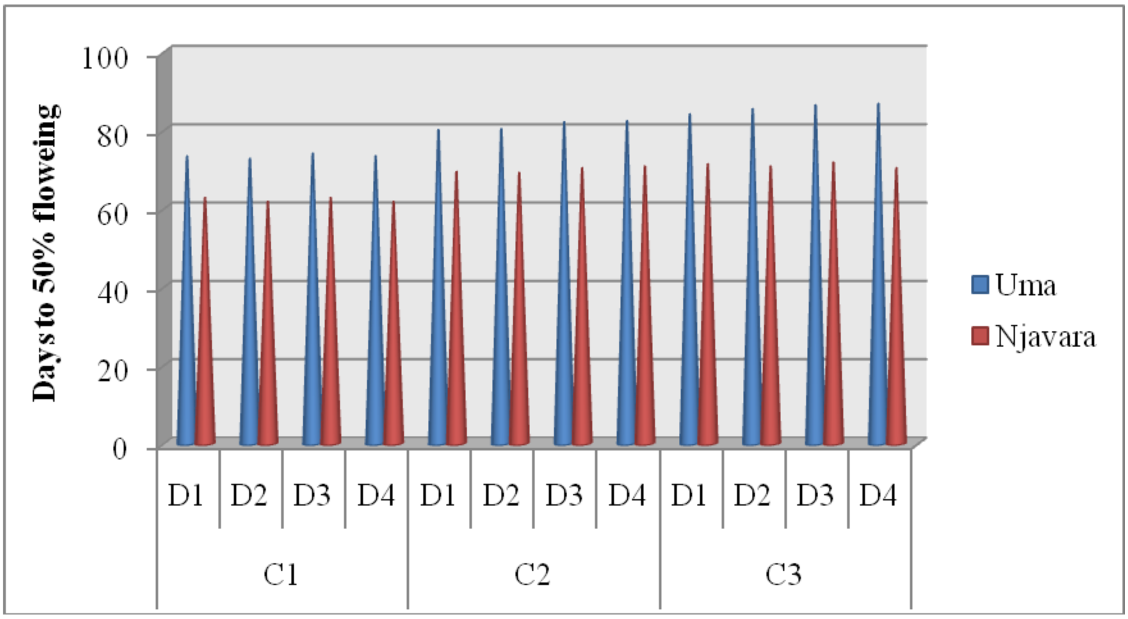


Figure3. Effect of elevated CO₂ and zinc treatments on No. of tillers of rice:

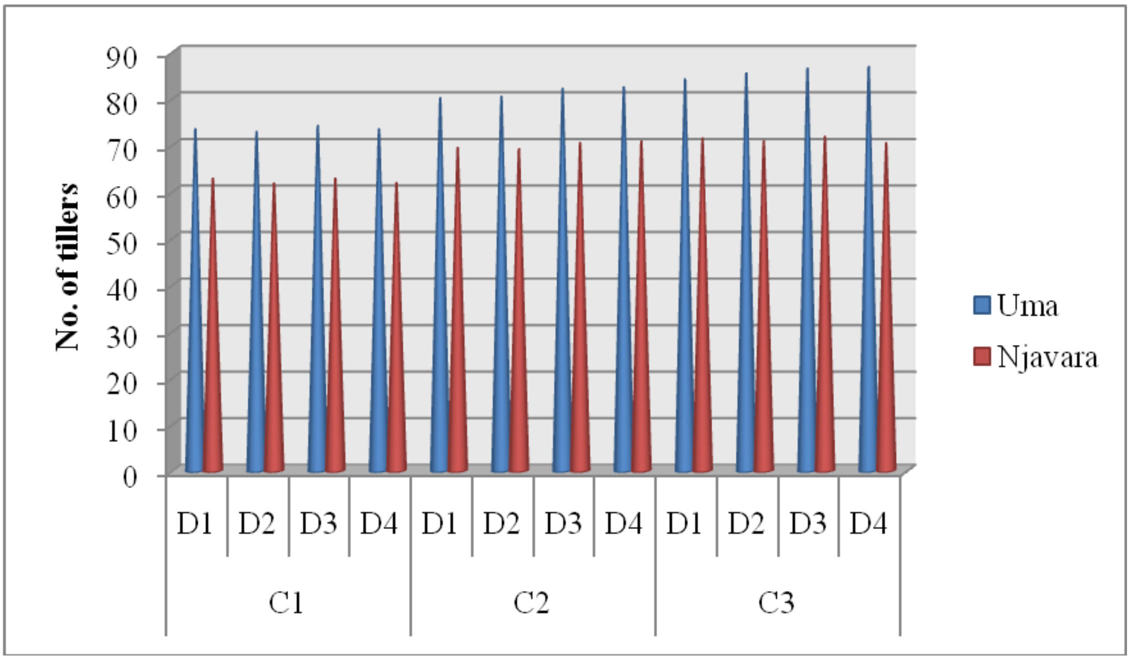


Figure4. Effect of elevated CO₂ and zinc treatments on root weight (g/plant) of rice:

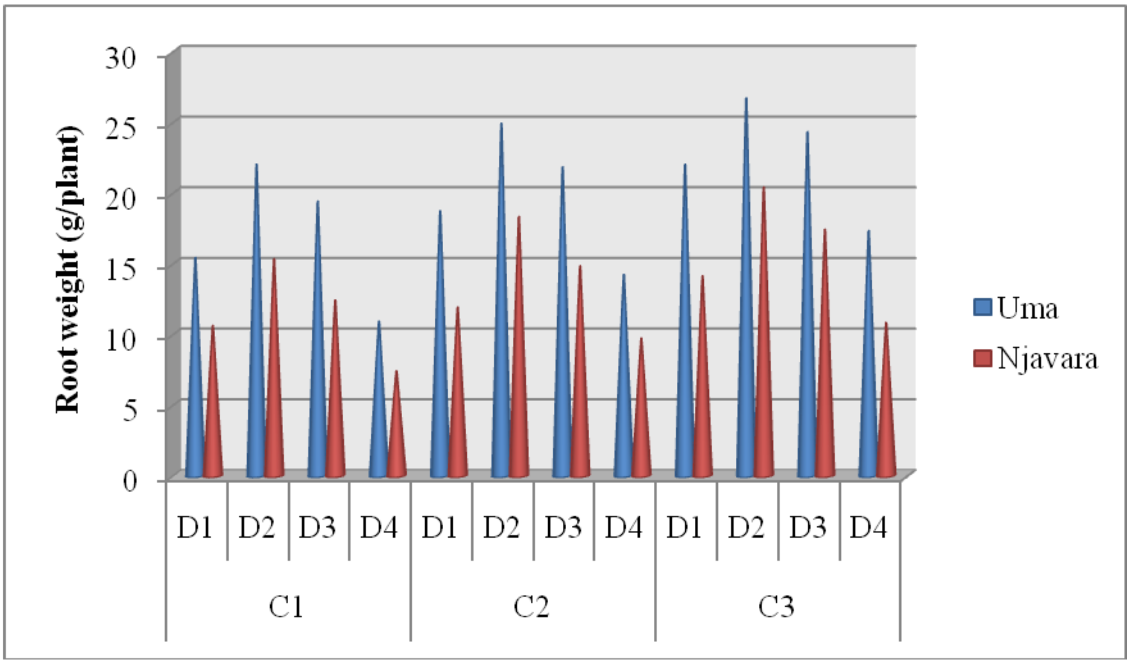


Figure5. Effect of elevated CO_2 and zinc treatments on straw yield (g/plant) of rice:

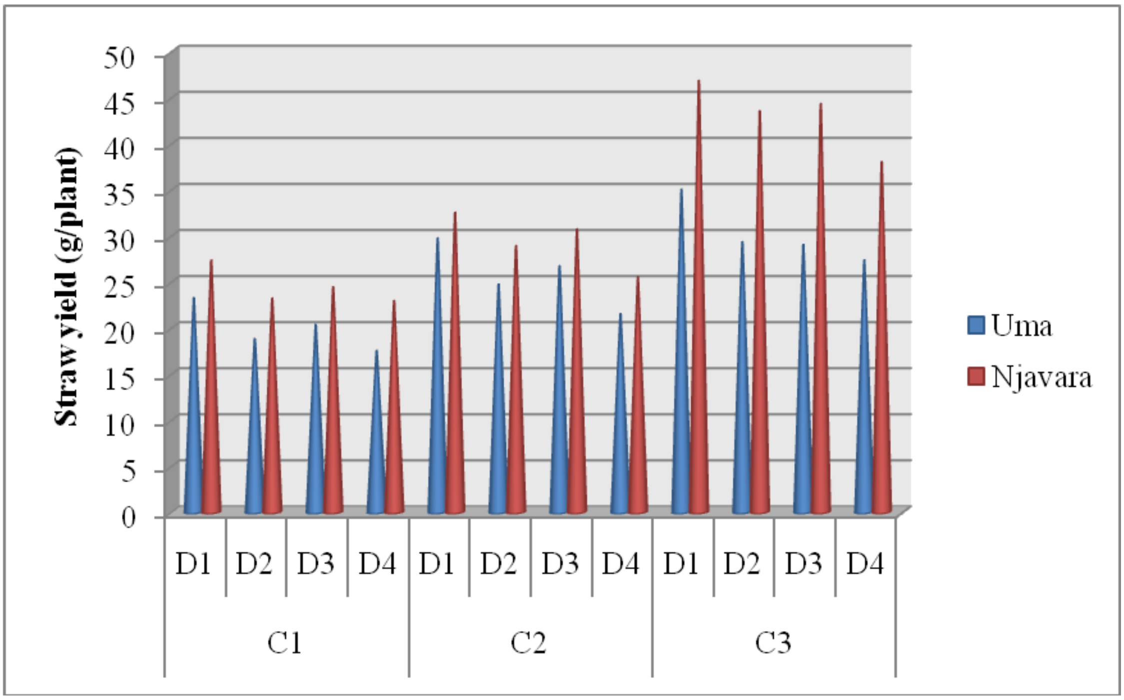


Figure6. Effect of elevated CO_2 and zinc treatments on total chlorophyll content of rice at active tillering stage (mg g^{-1} fresh weight):

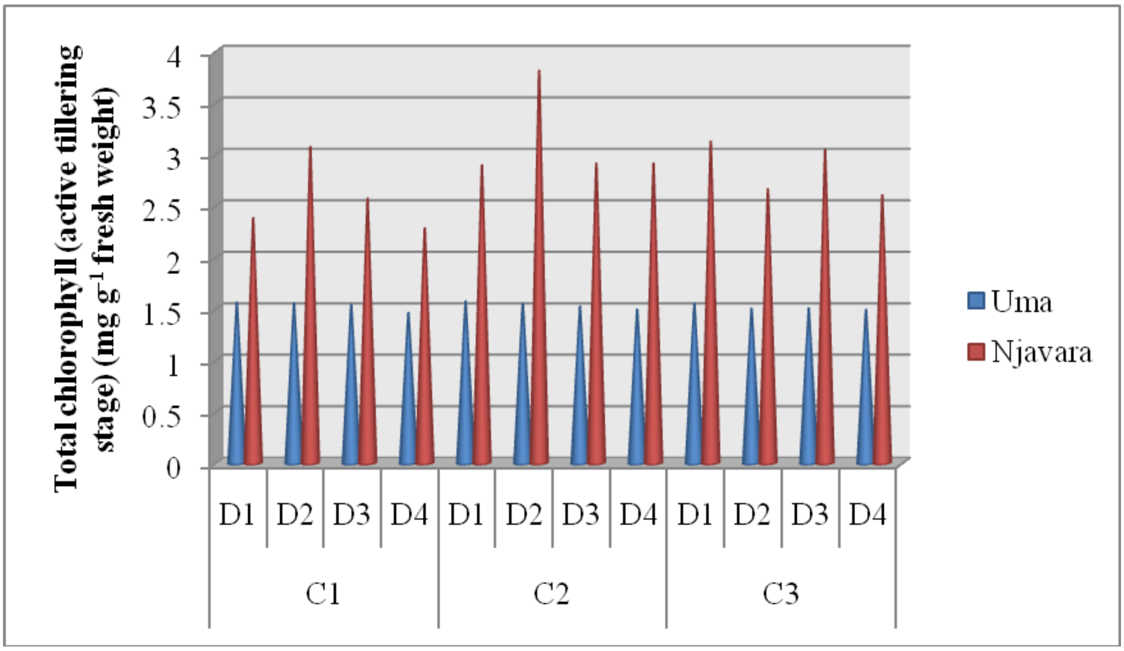


Figure7. Effect of elevated CO_2 and zinc treatments on total chlorophyll content of rice at grain filling stage (mg g^{-1} fresh weight):

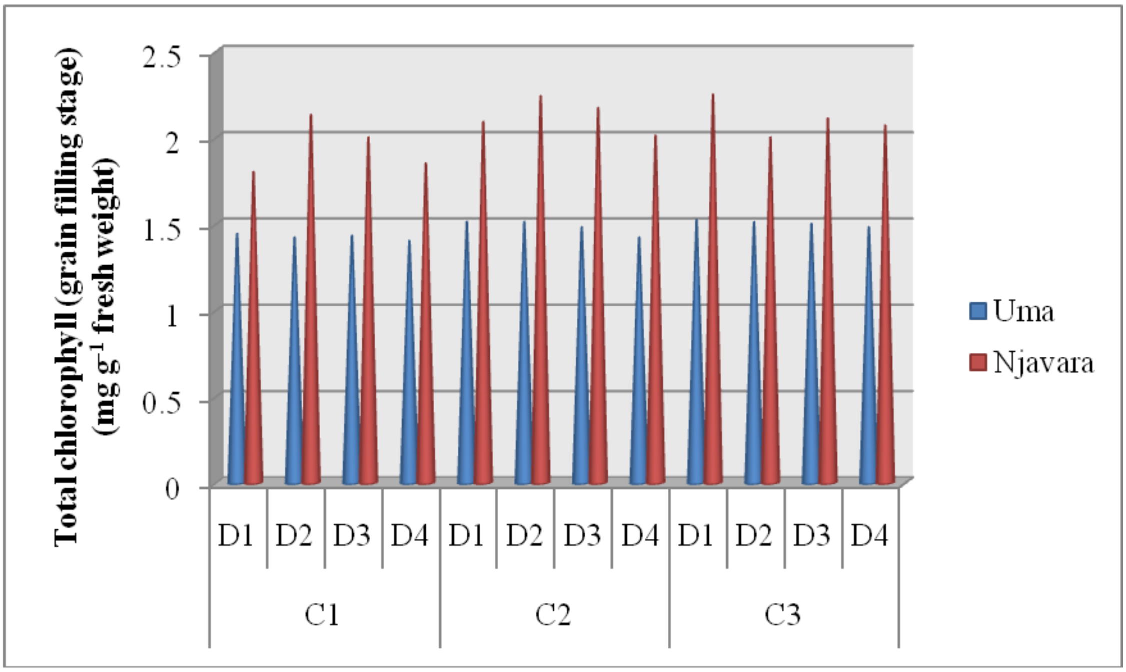


Figure8. Effect of elevated CO_2 and zinc treatments on total soluble protein content of rice at active tillering stage (mg g^{-1}):

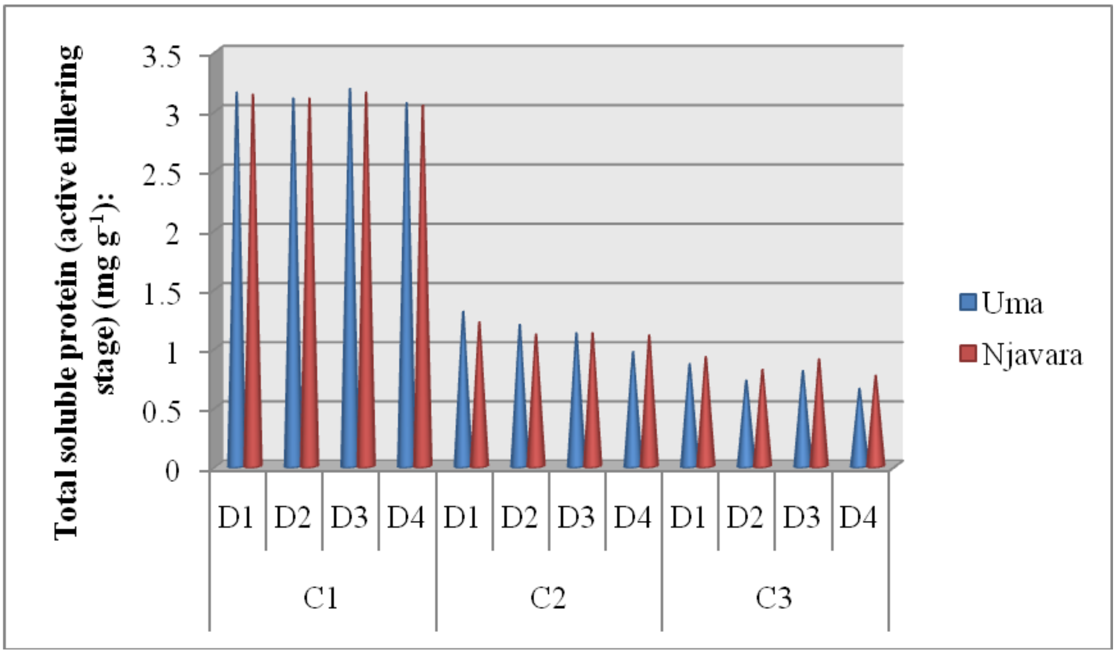


Figure9. Effect of elevated CO_2 and zinc treatments on total soluble protein content of rice at grain filling stage (mg g^{-1}):

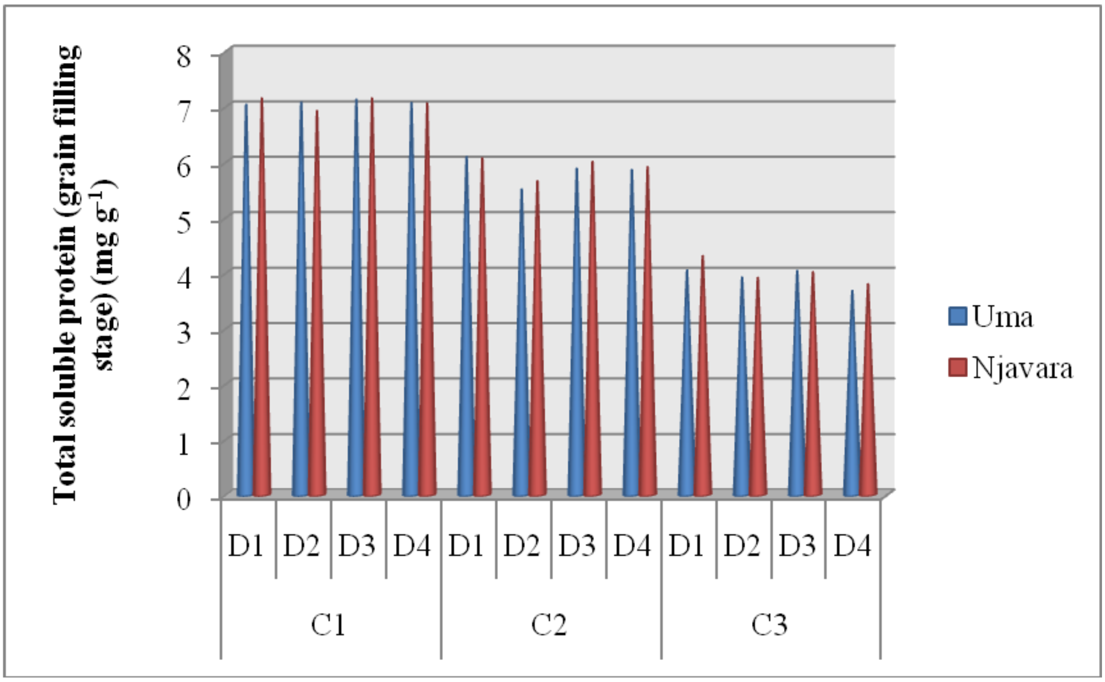


Figure10. Effect of elevated CO_2 and zinc treatments on total reducing sugars of rice at active tillering stage (mg g^{-1}):

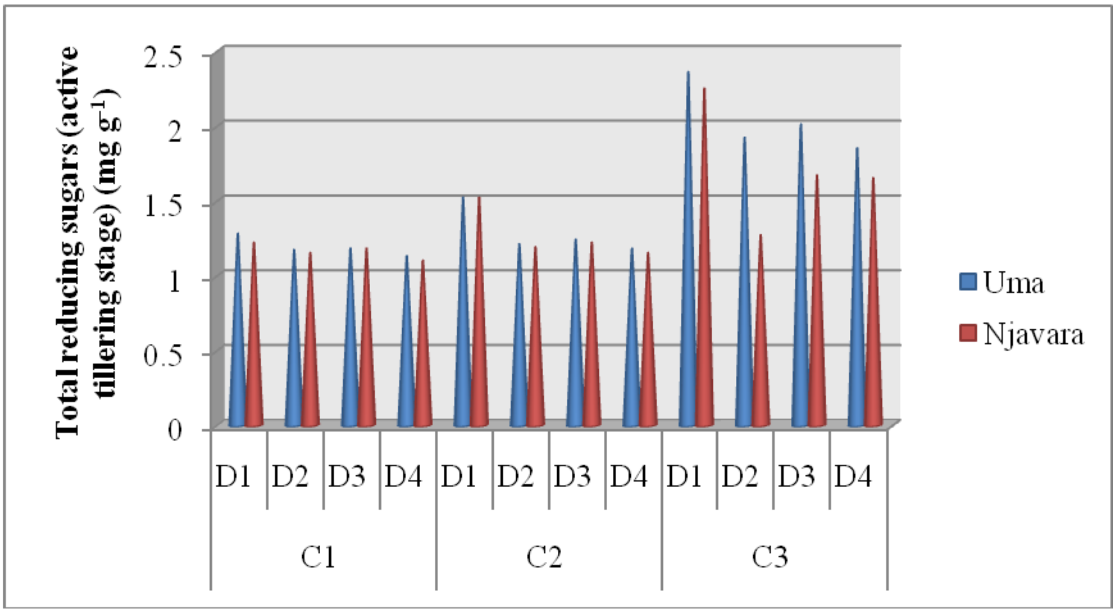


Figure11. Effect of elevated CO_2 and zinc treatments on total reducing sugars of rice at grain filling stage (mg g^{-1}):

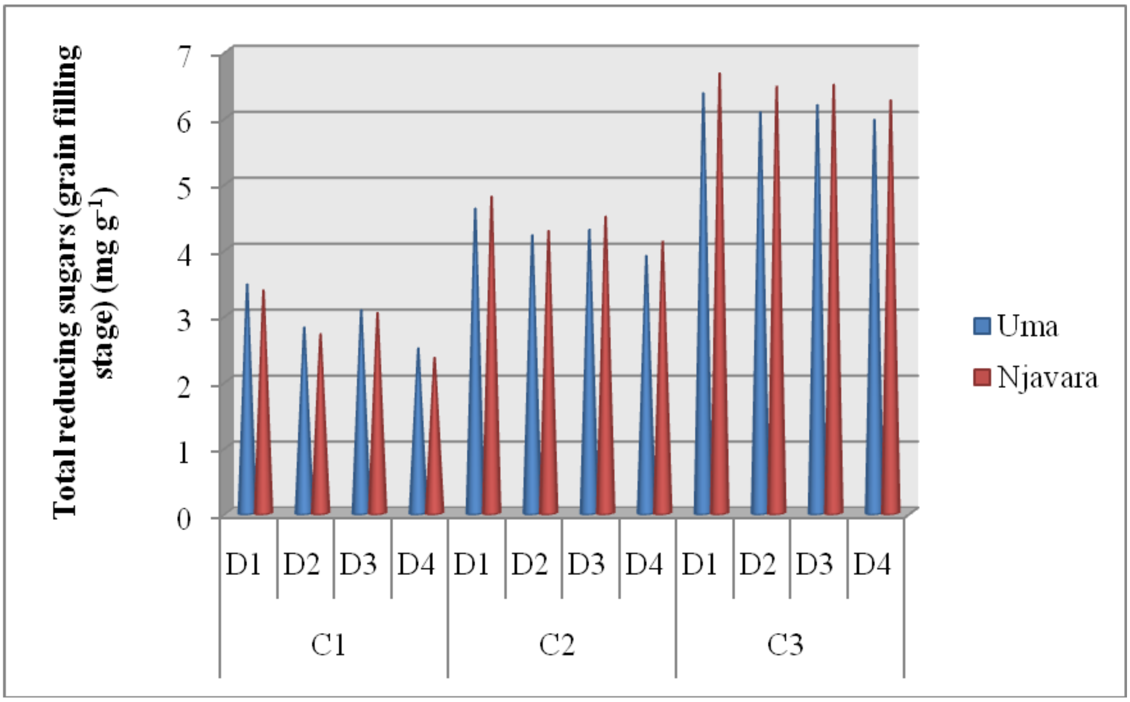


Figure12. Effect of elevated CO_2 and zinc treatments on photosynthetic rate of rice at active tillering stage (µmol CO_2 m $^{-2}s^{-1}$):

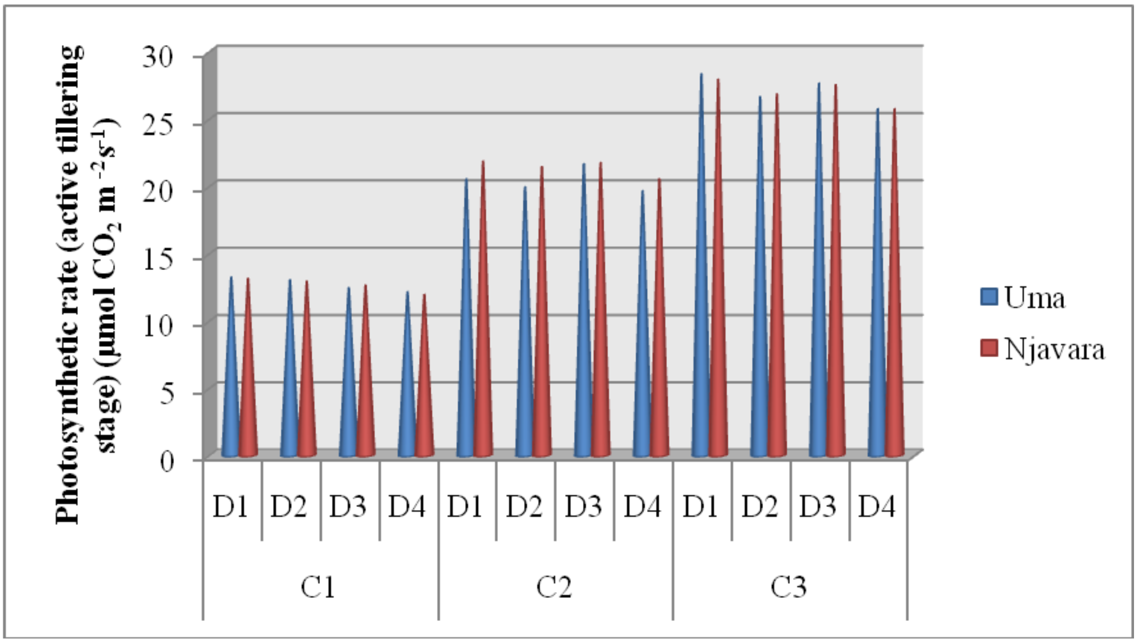


Figure13. Effect of elevated CO_2 and zinc treatments on photosynthetic rate of rice at grain filling stage (µmol CO_2 $m^{-2}s^{-1}$):

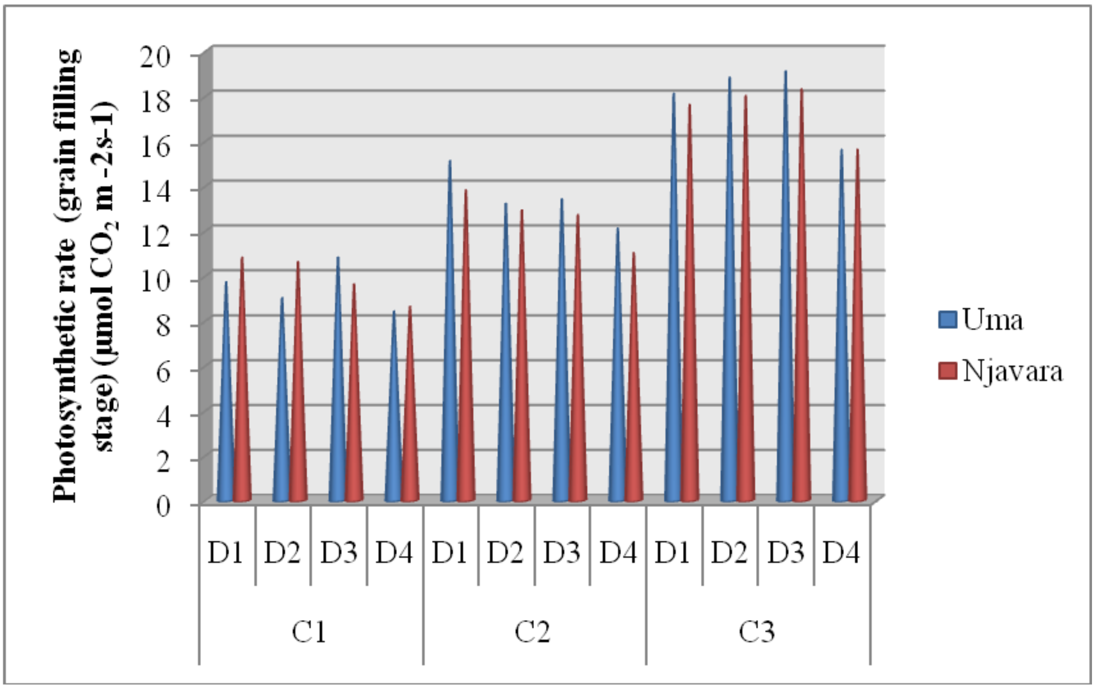


Figure14. Effect of elevated CO_2 and zinc treatments on transpiration rate of rice at active tillering stage (mmol H_2O m^{-2} s^{-1}):

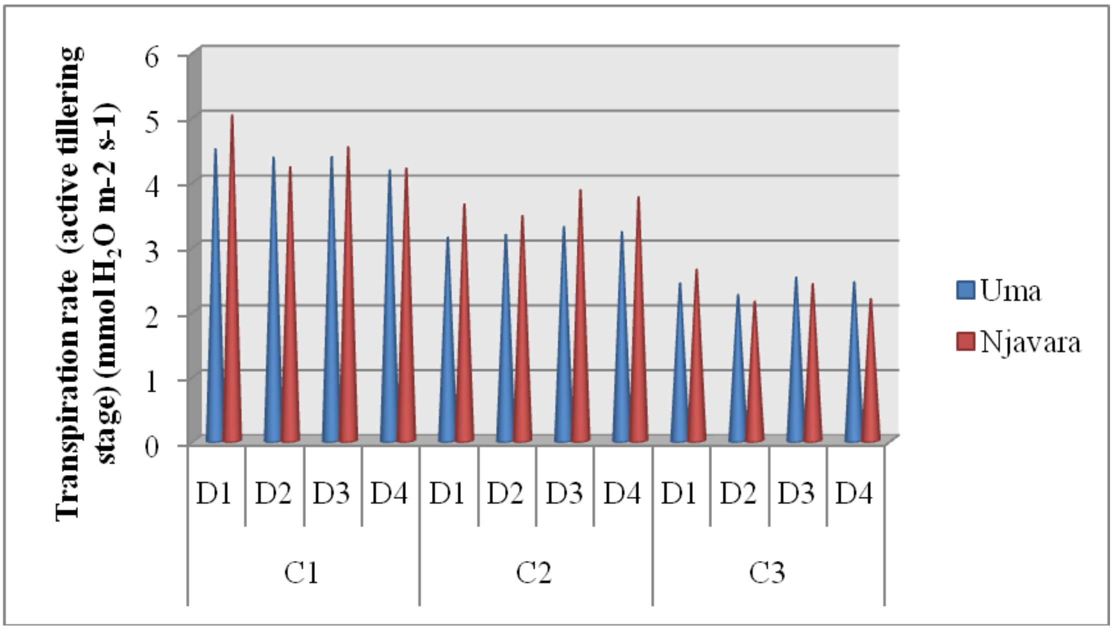


Figure15. Effect of elevated CO_2 and zinc treatments on transpiration rate of rice at grain filling stage (mmol H_2O m^{-2} s^{-1}):

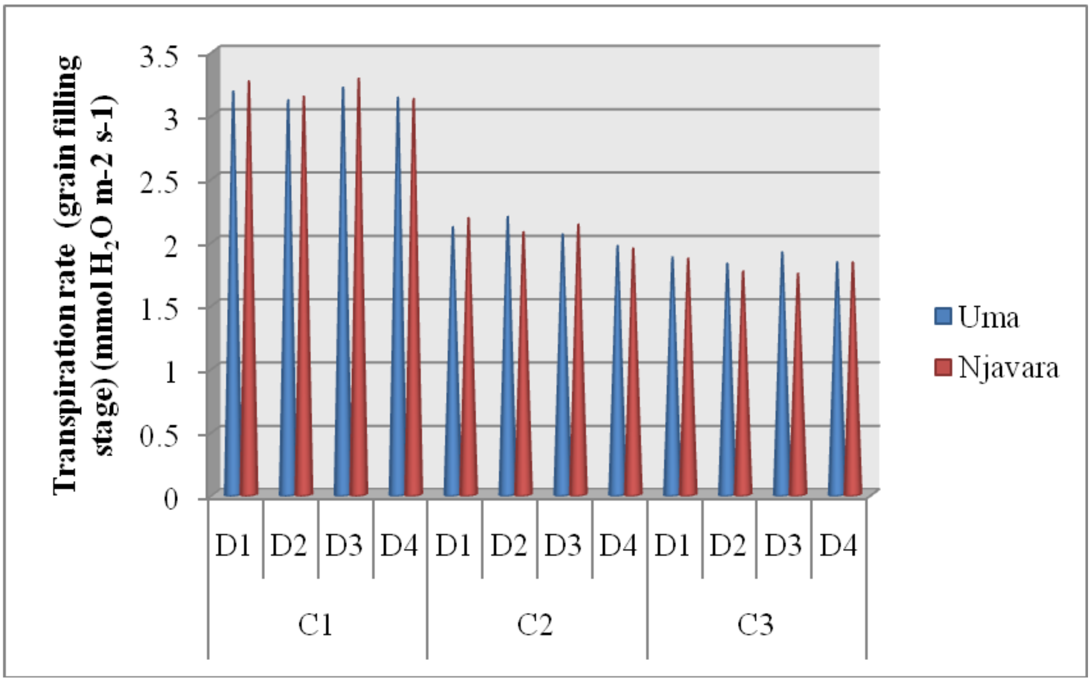


Figure16. Effect of elevated CO_2 and zinc treatments on stomatal conductance of rice at active tillering stage (mmol H_2O m^{-2} s^{-1}):

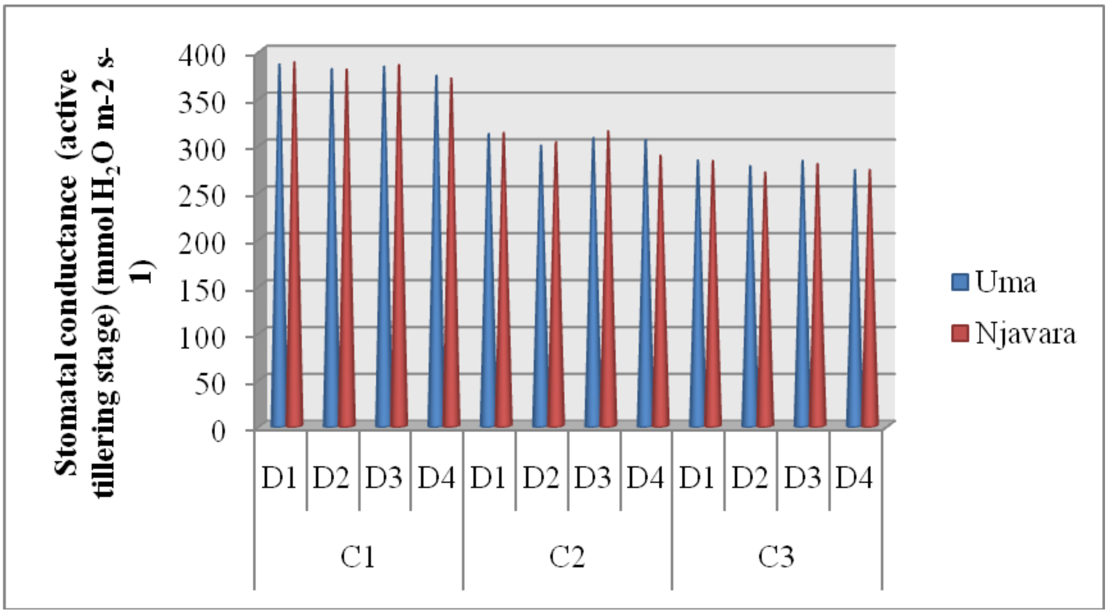


Figure15. Effect of elevated CO_2 and zinc treatments on transpiration rate of rice at grain filling stage (mmol H_2O m^{-2} s^{-1}):

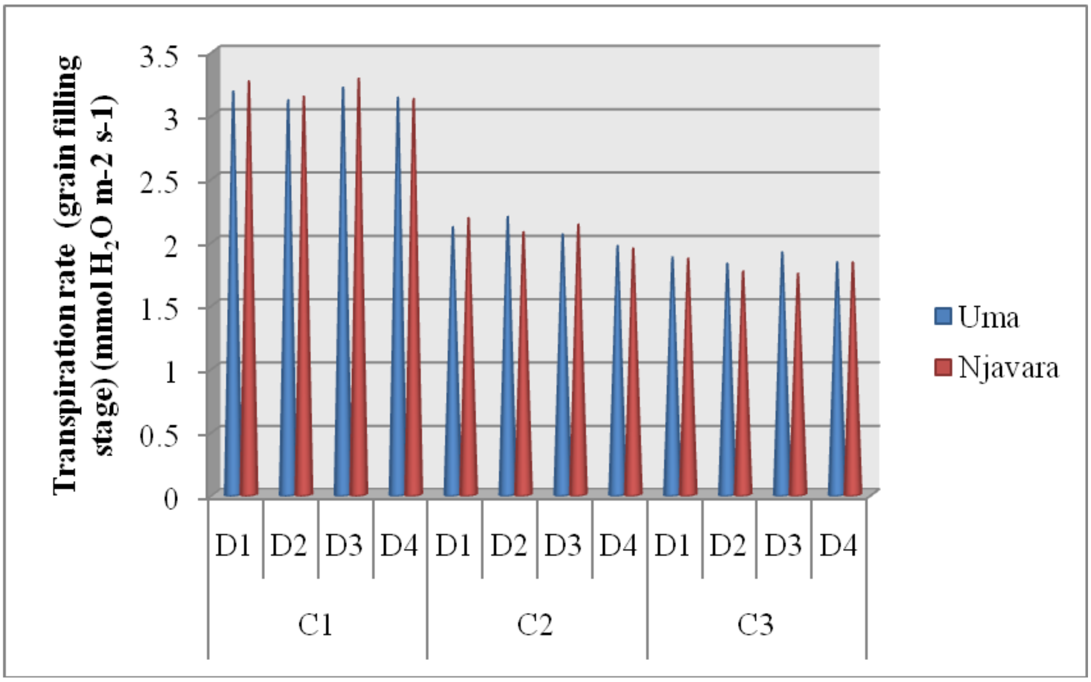


Figure16. Effect of elevated CO_2 and zinc treatments on stomatal conductance of rice at active tillering stage (mmol H_2O m^{-2} s^{-1}):

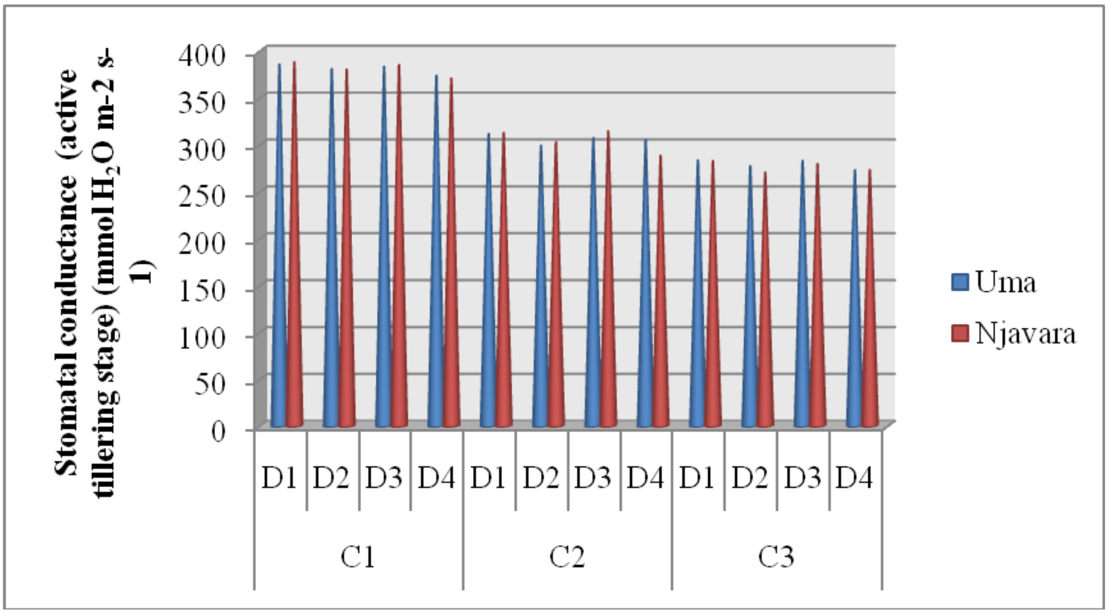
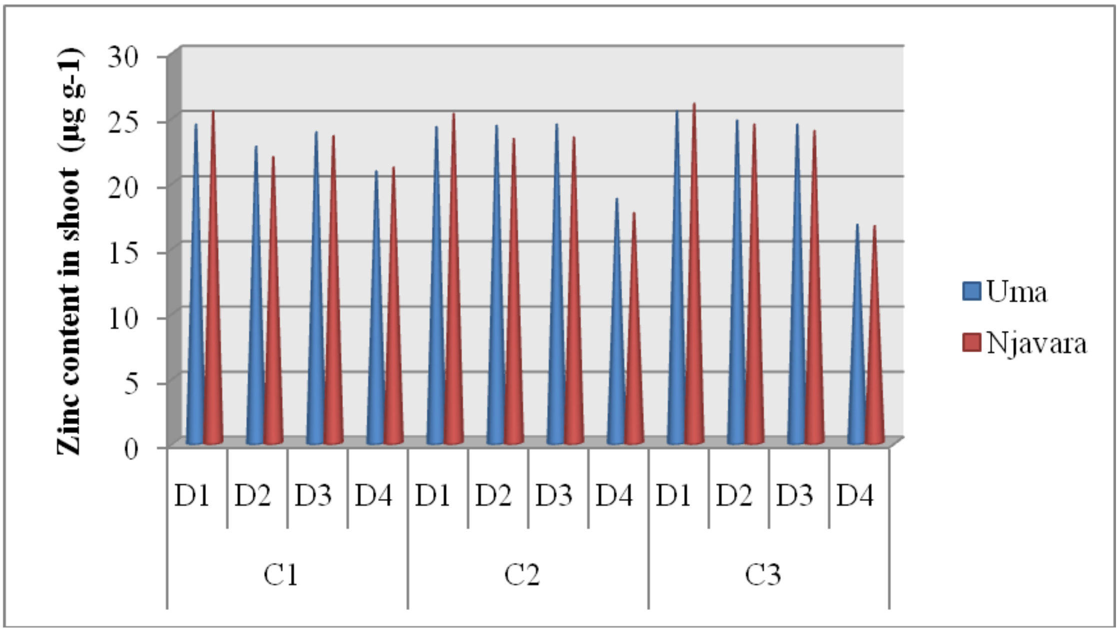


Figure19. Effect of elevated CO_2 and zinc treatments on zinc content in shoot of rice (µg g^{-1}):



Summary

6. SUMMARY

Zinc (Zn) is an essential mineral nutrient for all organisms. It is required as a cofactor in over 300 enzymes and plays critical structural roles in many proteins. Zn deficiency is known to show serious impact on human health, particularly in children, such as impairments in physical growth, immune system, learning ability and causing DNA damage and cancer development (Black *et al.,* 2008). Zn deficiency in human beings is prevalent and is envisaged to affect about 25% of the global population (Maret and Sandstead, 2006). WHO (2002) reported that Zn deficiency occupies fifth rank among the most important health risk factors in developing countries and eleventh globally.

Cereals are the major sources of Zn that are cultivated for their edible grains. Rice (*Oryza sativa* L.) is the worlds' most important cereal and staple food crop for more than half of the global population. It supplies more energy in the form of calories and is a good source of thiamine, riboflavin, and niacin and is an essential source of Zn for the poor residing in rural areas (Jiang *et al*., 2008).

Global climate change is considered to be the most serious environmental threat that the world is facing. It has been the centre of debate among the global scientific community recently. The major factor contributing to this climate change is the increasing greenhouse gases, especially CO_2 which is contributing to global warming. Concentration of CO_2 has increased from the pre-industrial level of 300ppm to the current level of 411ppm as of June, 2019 in the atmosphere. Higher concentrations of CO_2 are generally known to stimulate plant photosynthesis and growth although, recent research suggests that predicting its impacts on plant growth and development is complex. However, information on the eCO_2 effects on nutritional quality in relation to major nutrients and therefore the health-promoting phytochemicals in food crops indicate that major nutrients in food crops including protein, phosphorus, potassium, calcium, iron, Zn and other micronutrients in many food crops are reduced at eCO_2 levels (Rajashekar, 2018). For several decades, multiple experiments examining the response of food crops in controlled indoor environments have found significant losses in the concentration of Zn important for human health. This was also reported in the case of wheat, barley and rice grown in controlled-environment chambers (Myers *et al*., 2014). In turn, this is able to menace human nutrition, particularly in low-resource countries where people rely on mostly on cereals for their micronutrient intake.

In this scenario, attempts are to be made to evaluate the performance of popular rice varieties of Kerala under increasing CO_2 condition and also to enrich Zn status of grains. In this context, the present study "Influence of elevated CO_2 on Zn dynamics in rice (*Oryza sativa* L.)" was undertaken with the objective of assessing the impact of eCO_2 on growth, development and nutritional quality of rice in relation to modifications in Zn dynamics.

The rice genotypes- Uma (MO16) and Njavara-golden yellow which were collected from IFRI, Karamana and RARS, Pattambi respectively were maintained under three CO_2 conditions i.e., 500 ppm in OTC (Open Top Chamber), 420 ppm under Polyhouse and 390 ppm under open field condition with four Zn enrichment treatments (D_1- Foliar spray ($ZnSO_4$ 0.5% at panicle initiation and grain filling stage); D_2-AMF (3g of AMF inoculum /cavity of protrays); D_3-Seed treatment: seed priming with 6.4% $ZnSO_4$ for 36 hr and D_4-No treatment). The experiments were laid out in CRD with 24 treatment combinations and three replications.

The experiment has shown increased physio-morphological and biochemical traits under OTC followed by Polyhouse relative to open field condition. The treatment with 0.5% $ZnSO_4$ foliar spray (D_1) recorded highest plant height (102cm), number of tillers (34) and straw yield, (35g/plant) in Uma and in Njavara the same treatment resulted in highest plant height (130cm), number of tillers (32) and straw yield (46.8g/plant). AMF treatment (D_2) has recorded highest root weight both in Uma (26.7g/plant) and Njavara (20.4g/plant). The number of days to 50% flowering was increased under OTC (85 days in Uma and 71 days in Njavara) and polyhouse (80 days in Uma 69 days in Njavara) as against 72 days in Uma and 61days in Njavara under open field condition.

Physiological and biochemical parameters such as total chlorophyll content, photosynthetic rate and total reducing sugars which were recorded at active tillering and grain filling stage were also significantly higher in the case of plants kept under OTC condition followed by those under polyhouse and control condition. OTC condition has shown highest values at active tillering stage in total chlorophyll content (2.33 mg g^{-1} fresh weight), photosynthetic rate (27 µmol CO_2 $m^{-2}s^{-1}$) and total reducing sugars (1.9 mg g^{-1}). The same trend was observed in grain filling stage in total chlorophyll content (1.805 mg g^{-1} fresh weight), photosynthetic rate (17.6 µmol CO_2 $m^{-2}s^{-1}$) and total reducing sugars (6.3 mg g^{-1}). However the photosynthetic rate and total chlorophyll content were decreased at grain filling stage in all the three CO_2 conditions.

All the Zn treatments had significant influence on all the above parameters except photosynthetic rate. Foliar spray with 0.5% $ZnSO_4$ (D_1) recorded highest total chlorophyll content (1.52 mg g^{-1} fresh weight), and total reducing sugars (2.25 mg g^{-1}) in Uma and 2.89mg g^{-1} fresh weight of total chlorophyll content, 2.25 mg g^{-1} of total reducing sugars in Njavara at active tillering stage under OTC condition. The same trend was obtained at grain filling stage, with the highest total chlorophyll content (1.57 mg g^{-1} fresh weight), and total reducing sugars (6.36 mg g^{-1}) in Uma and 2.89mg g^{-1} fresh weight of total chlorophyll content, 6.66 mg g^{-1} of total reducing sugars in Njavara.

Some physiological and biochemical parameters such as total soluble protein, transpiration rate and stomatal conductance were decreased under increased levels of CO_2. At active tillering stage lowest values were recorded in total soluble protein (0.8 mg g^{-1}), transpiration rate (2.4 mmol H_2O $m^{-2}s^{-1}$) and stomatal conductance (277.2 mmol H_2O $m^{-2}s^{-1}$) under OTC. At grain filling stage 7.1 mg g^{-1} of total soluble protein, 1.8 mmol H_2O $m^{-2}s^{-1}$ of transpiration rate, 226 mmol H_2O $m^{-2}s^{-1}$ of stomatal conductance were recorded. Control plants recorded the highest total soluble protein content at active tillering stage and grain filling stage in both the varieties. The transpiration rate and stomatal conductance did not show significant response to Zn treatments in both the varieties.

Yield attributes and Zn content in grain and bran were not recorded in OTC because of failure in grain development. Due to the increase in mean daily air temperature to 40°C at grain filling stage, the grains turned out to be chaffy. parameters related to Zn dynamics were analyzed in leaf and shoot under all the conditions. Decreasing levels of Zn in leaf and shoot with increasing levels of CO_2 were evident in both the varieties of rice in the present study. Though all the Zn treatments were having positive influence on Zn status, foliar application of 0.5% $ZnSO_4$ was found to be the most effective one. The Zn content in leaf was highest in control plants with 25.8 $\mu g\ g^{-1}$ in Uma and 26.1$\mu g\ g^{-1}$ in Njavara. Similarly, the Zn content in shoot was also recorded highest in the control plants with 25.4 $\mu g\ g^{-1}$ in Uma and 26 $\mu g\ g^{-1}$ in Njavara.

All the treatments used for Zn enrichment viz., foliar spray ($ZnSO_4$ 0.5% at panicle initiation and grain filling stage), AMF (3g of AMF inoculum /cavity of protrays) and seed priming with 0.64% $ZnSO_4$ for 36 hrs were effective in improving Zn status under all the three conditions including Open Top Chamber , polyhouse and open field condition. Among them, foliar spray with 0.5% $ZnSO_4$ resulted in best result followed by seed priming and AMF treatment.

The present investigation has shown a positive influence of increasing CO_2 concentrations on growth and dry matter accumulation in rice varieties; Uma (MO16) and Njavara-golden yellow. Increasing CO_2 concentrations was also found to modify the Zn dynamics in both the rice varieties with a negative influence on Zn contents of leaf and shoot tissues which could significantly be improved by foliar spray of 0.5% $ZnSO_4$ solution at panicle initiation and grain filling stages.

The information generated proposes the possibility of utilizing low-cost poly houses for maintaining moderately higher levels of CO_2 concentrations along with foliar application of $ZnSO_4$ for improving the yield and nutritional status of rice. The research outcome can also be helpful to standardize agricultural remedies for addressing nutritional problems associated with the increasing atmospheric CO_2 concentration.

Further steps should also be taken up towards understanding modifications in Zn concentrations in different plant parts including endosperm and bran under increasing CO_2 levels. Physiological and molecular mechanisms underlying the modifications in uptake, translocation and remobilization should also be addressed for identifying the critical candidate genes and their deployment in developing high Zn rice varieties.

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Appendices

APPENDICES

I. CHEMICALS FOR REDUCING SUGARS ESTIMATION

Dinitrosalicylic acid (DNS) reagent (100ml)

DNS	1 g
Crystalline phenol	200 mg
Sodium sulphite	50 mg
1% NaOH	1 g NaOH dissolved in 100ml distilled water

Final volume was adjusted to 100 ml

40% Rochelle salt solution	40 g Rochelle salt solution dissolved in 100ml distilled water

II. CHEMICALS FOR TOTAL CHLOROPHYLL ESTIMATION

80% Acetone: DMSO (1:1 v/v)

80% Acetone	80 ml Acetone is taken and volume made to 100ml using distilled water
Dimethyl sulfoxide (DMSO)	100 ml

III. CHEMICALS FOR TOTAL SOLUBLE PROTEIN ESTIMATION

Phosphate buffered saline (PBS) (100ml)

0.2M Na_2HPO_4 (Solution A)	28.392g dissolved in 1000ml distilled water
0.2M NaH_2PO_4 (Solution B)	23.996g dissolved in 1000ml distilled water

51 ml from solution A and 49ml from solution B is mixed and made to 100ml and the pH is adjusted to 7.8.

Bradford reagent (200ml)

Comassie brilliant blueG 250	100 mg dissolved in 50ml of 95% ethanol
Ortho phosphoric acid	100ml

Final volume adjusted to 200ml

Abstract

INFLUENCE OF ELEVATED CO_2 ON ZINC DYNAMICS IN RICE

(Oryza sativa L.)

by

Ramireddy Bhavana

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ABSTRACT

The present programme “Influence of elevated CO_2 on zinc dynamics in rice (*Oryza sativa* L.)” was undertaken in the Department of Plant Physiology, College of Agriculture, Vellayani during 2018-2020 with the objective of assessing the impact of elevated CO_2 on growth, development and nutritional quality of rice in relation to modifications in Zn dynamics.

The extent of variation in growth, physiological, biochemical parameters and parameters related to Zn dynamics were assessed in two rice genotypes, Uma (MO16) and Njavara-golden yellow. Plants were maintained under three CO_2 conditions i.e., OTC (Open Top Chamber) (500 ppm), Polyhouse (420 ppm) and open field condition (390 ppm) with four zinc enrichment treatments (D_1- Foliar spray ($ZnSO_4$ 0.5% at panicle initiation and grain filling stage); D_2-AMF (3g of AMF inoculum /cavity of protrays); D_3-Seed treatment: seed priming with 6.4% $ZnSO_4$ for 36 hrs; D_4-control) with the aim to improve the zinc status of rice plants. The experiments were laid out in CRD with 24 treatment combinations and three replications.

The study indicated that upon exposure to 500ppm CO_2, highest values were recorded in plant height, no. of tillers, root weight and straw yield in the case of both varieties. The treatment with 0.5% $ZnSO_4$ foliar spray (D_1) recorded highest plant height (102cm), no. of tillers (34) and straw yield, (35g/plant) in Uma and in Njavara the same treatment resulted in highest plant height (130cm), no. of tillers (32) and straw yield (46.8g/plant). AMF treatment (D_2) has recorded highest root weight both in Uma (26.7g/plant) and Njavara (20.4g/plant). The no. of days to 50% flowering was increased under OTC (85 days in Uma and 71 days in Njavara) and Polyhouse (80 days in Uma 69 days in Njavara) as against 72 days in Uma and 61days in Njavara under open field condition.

Physiological and biochemical parameters recorded at active tillering and grain filling stage had shown that OTC condition resulted in highest total chlorophyll content (2.33 mg g^{-1} fresh weight), photosynthetic rate (27 µmol CO_2 $m^{-2}s^{-1}$) and total reducing sugars (1.9 mg g^{-1}) at active tillering stage. The same trend was observed in

grain filling stage also but to a lesser extent in total chlorophyll content (1.805 mg g^{-1} fresh weight) and photosynthetic rate (17.6 μmol CO_2 $m^{-2}s^{-1}$) whereas the total reducing sugars (6.3 mg g^{-1}) was increased to a greater extent. However the photosynthetic rate and total chlorophyll content were decreased at grain filling stage in all the three CO_2 conditions. Lowest total soluble protein was recorded under OTC in active tillering stage (0.8 mg g^{-1}) and grain filling stage (7.1 mg g^{-1}). All the Zinc treatments had significant influence on all the above parameters and foliar spray with 0.5% $ZnSO_4$ resulted in maximum enhancement. The transpiration rate and stomatal conductance which was decreased under eCO_2 did not show significant difference upon application of Zn treatments in both the varieties.

Yield attributes and Zn content in grain and bran could not be recorded as there was no grain development in plants under OTC due to high temperature. The parameters related to zinc dynamics were analyzed in leaf and shoot under all the conditions. Decreasing levels of zinc in leaf and shoot with increasing levels of CO_2 was evident in both the varieties of rice in the present study in untreated plants. Though all the zinc treatments were having positive influence on Zinc status, foliar application of 0.5% $ZnSO_4$ was found to be the most effective one followed by seed treatment and AMF. The zinc content in leaf was highest in D_1 with 25.8 μg g^{-1} in Uma and 26.1μg g^{-1} in Njavara. Similarly, the zinc content in shoot was also recorded highest in D_1 with 25.4 μg g^{-1} in Uma and 26 μg g^{-1} in Njavara followed by D_3 and D_2.

In the present study, increasing concentration of CO_2 was found to enhance growth and development in rice variety Uma and Njavara by inducing increased photosynthetic rate, chlorophyll and reducing sugars content but at the same time it had a negative influence on Zinc content leaf and shoot tissue which was decreased due to the accumulation of more no. of carbohydrates which has diluted the Zn concentration in the leaf and shoot tissue in the untreated plants. Supplement of zinc through different treatments has elevated the zinc content in the leaf and shoot tissue in all the three different CO_2 conditions.

These results offer a small slice of the broad implications for the impact of higher CO_2 on nutrition and health. Taken together, CO_2 induced nutritional declines could produce a major headwind on progress towards alleviating malnutrition and deserves attention and concerted action.

The information generated will help farmers to raise quality produce with the help of low cost technologies, to make paddy cultivation more profitable and can also be an answer to the serious health crisis and malnutrition faced globally in this changing climatic scenario.