Differential physiological response of nitrogen-efficient and nitrogen-inefficient rice varieties to elevated CO_2 level and low nitrogen

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Abstract: In the present investigation, effect of elevated CO2 on the growth and photosynthesis of nitrogen-inefficient (cv. Rasi) and nitrogen-efficient (cv. CR Dhan) rice varieties was studied. The rice varieties were grown in the growth chamber under the treatments of elevated CO2 and low-nitrogen (N). Ambient CO2 level and optimum N treatment was maintained as control. Forty-five-day old plants were harvested for the measurement of growth parameters. Photosynthesis and related parameters were measured in the intact 45-day-old plants. Low-N treatment under the condition of ambient CO2 reduced the growth and photosynthesis significantly in both the varieties, when compared to control. However, the level of reduction was higher in cv. Rasi than cv. CR Dhan. Elevated CO2 treatment at optimum N condition increased the growth and photosynthesis of both the varieties significantly, compared to control. However, under the condition of low N, elevated CO2 level reduced the growth and photosynthetic capacity of rice varieties. Between the two varieties, the reduction in the growth was higher in cv. Rasi. It has been suggested that the nitrogen efficient variety of rice can grow well under the conditions of low N supply and elevated CO2 level.

Key words- Nitrogen, elevated CO_2, rice, growth

I. INTRODUCTION

Rice (Oryza sativa L.) is an unambiguously important food crop; half of the population of the world relies on this for their food. Carbon dioxide is an indispensable inorganic substrate for plant growth and development. The global atmospheric CO2 concentration is projected to reach the level of 550-700 ppm within this century (Prentice et al., 2001). This increase in atmospheric [CO_2] has the potential to enhance the growth and yield of many agricultural crop species because CO_2 is needed for plant photosynthesis (Stitt and Krapp, 1999). However, a doubling of CO_2 level initially accelerates carbon fixation in C3 plants by about 30%. Yet, after days to weeks of exposure to high [CO_2], carbon fixation declines until it stabilizes at a rate that averages 12% about ambient controls. This general phenomenon known as CO_2 acclimation of carboxylation capacity is correlated with a decline in the activated rubisco another enzyme in the Calvin cycle. The change in the Calvin cycle enzyme activity is correlated with decline in overall shoot protein and nitrogen content (Imai et al., 2008). Models feature in the third assessment report of the IPCC suggest that increasing atmospheric CO_2 alone causes 350-890 Pg of carbon to accumulate in the terrestrial biosphere by 2100. This CO_2 projection requires 7.7-37.5 Pg of nitrogen by 2100. Combining all high estimates, the accumulation of nitrogen will be 6.1 Pg. Low estimates of nitrogen accumulation yield only 1.2 Pg nitrogen. This amount of nitrogen accumulation is less than the required by all CO_2 simulations. These models and experiments suggested that under elevated CO2 conditions and limited nitrogen supply, there will be decline in crop productivity. Nitrogen limited plants are more suitable as comparison to nitrogen sufficient plants for acclimation of photosynthesis to elevated CO_2 (Bowler and Press 1996). This is also investigated that the carboxylation capacity of limited nitrogen plants is more as compared to nitrogen-rich plants.

Nitrogen is another vital element for plant growth and development. The mechanism of nitrogen consumption is tightly associated with carbon consumption. The growth of the plant is impossible without the essential nutrients, and N is one of them, required from germination to maturity during the development of the plant. N limitation not only effects at the physiological level of the plant but also at the molecular level. For the past half-century, the increase in crop production is achieved by the application of N fertilizers; however, only a percentage of about 30 to 40 is utilized by the plant, and the remaining portion may leach out or go through the geochemical cycle (Hakeem et al. 2012). The use of nitrogen fertilizer increases with the increasing demand of food for the growing population.

Crop productivity is directly associated with the application of nitrogen fertilizer. The consumption of nitrogen fertilizer increased 100 times in the last 100 years (Ladha et al., 2005). But the used nitrogen is not completely utilized by the crop, so the problem started from here. Since nitrogen utilization efficiency by the crop at the agriculture field is 30%-40% according to previous report rest of 60%-70% unutilized nitrogen fertilizer creates severe problems like related to environment and health hazards (Jeffrey et al. 2002). In India, the consumption of nitrogen fertilizer increases tremendously in the last fifty years (Pathak et al. 2016). In 1950 the consumption of nitrogen was only 0.5 million tonnes that have increased to 15.6 million tonnes in 2015-16. With the current rate of nitrogen fertilizer consumption, the requirement of nitrogen will be 20-25 tonnes per year till the end of 2020. It has been evaluated that 100Tg of nitrogen is released every year from the agriculture field in the atmosphere.

Conversely, the high use of N fertilizers for the improvement of the crop may also have some damaging effects on the environment. It can be through eutrophication of nearby water bodies and thereby affecting the aquatic life of the habitat (Beman et al. 2005). Delayed flowering and prolong maturation time in some cases can, however, be induced by the application of nitrogen fertilizers, and may significantly increase the risk of yield losses, more significantly in the regions of high latitude where late-season low temperature would rigorously restrict the grains filling (Li et al. 2017). Therefore, one of the major prerequisites for the intensification of crop production in higher latitude regions is the early flowering and ultimately early maturation in shorter growing seasons (Izawa 2007). Recently, in the field of crop breeding, the most anticipated aim is to increase the yields by improving the nitrogen use efficiency (NUE) and thereby concurrently reducing the maturation times (Li et al. 2017), however, till date the there is very little success which is achieved by breeders in this field.

The present study was conducted to find out the changes in the growth and physiological parameters of two rice cultivars viz., CR Dhan 311 (N-efficient) and Rasi (N-inefficient) under low nitrogen and elevated CO2 conditions. This work will help to understand the physiological and photosynthetic regulation and a further improvement in the given attributes to sustain rice production at elevated CO2 and low nitrogen conditions.

II. MATERIALS AND METHODS

Rice (*Oryza sativa* L.) was used as experimental material. It belongs to the family poaceae. Rice plant is considered a semiaquatic annual grass with hollow, round, jointed culms, sessile leaf blade and a terminal panicle. The morphology of rice plant is divided in to vegetative phases (including germination, seedling, and tillering stages) and reproductive stages (including panicle initiation and heading stages). In our earlier experiment Rasi (V1) and CR Dhan 311 (V2) varieties of rice (*Oryza sativa* L.) have been identified as nitrogen-efficient and nitrogen-inefficient, respectively on the basis of various physiological and biochemical parameters (Tantay et al. 2019). Healthy seeds of these varieties were procured from Division of Agronomy, Indian Agriculture Research Institute, New Delhi (India). These were surface sterilized with 0.1 % HgCl2 for 1 min. and the seeds were then thoroughly rinsed with mineral free water 4-5 times. Sterilized seeds were pre-soaked for overnight in distilled water and then transferred at next morning into the pots. The plants were grown hydroponically in the nutrient solution (1/4th strength) in plant growth chamber. The growth chamber was maintained with 14/10 h light/dark, relative humidity of 60%, day/night temperature 22/28 °C and 430 µmol/m2/sec photosynthetic photon flux density.

Treatments

Different sets of treatment were designed for the showing the effect of different nitrogen and CO2 treatments. For the proper growth of rice, nutrient fertilizers were applied according to the recommended doses of CO2 and nitrogen (N). The recommended doses for N and CO2 were respectively. The first set of plants (T1) were grown at 100% of RDN (10 mM) and ambient CO2. The second set (T2) were grown at 50% RDN and ambient CO2. Third sets of plants (T3) were grown at sufficient nitrogen (10 mM) and elevated CO2 levels. Fourth set (T4) of plants were grown at low nitrogen (1 mM) and elevated CO2. Two sets of treatments were prepared. One set contain low nitrogen and optimum nitrogen with ambient CO2 (T1 and T2). Second set contains low nitrogen and optimum nitrogen with elevated CO2 (T3 and T4). Both the sets were placed in two chambers one chamber was with ambient CO2 and second chamber was with elevated CO2. The level of CO2 was maintained at 550 ppm using automated control device.

Collection of samples

Seeds were germinated after 4-5 days of sowing. Then they were transplanted after 26-28 days of sowing. Samplings were done at three stages viz., vegetative stage (6th tiller), flowering stage (booting stage), post flowering stage (panicle stage). Samplings were done at morning hours. For study of different growth, physiological and biochemical parameters, harvested leaves were taken and immediately dipped in liquid nitrogen and then wrapped in aluminum foils. After that for long term storage samples were labeled and kept in deep freezer at -80 ℃.

Determination of growth parameters

For determination of fresh weight of shoot, the plants were separated from roots and then washed with fresh water and dry then weight of leaves were measure by using electronic balance (Model BL2105, Sartorius, Germany). Root length was also measured by electronic balance. The length of root of shoot were measured by taking fresh leaves in morning hours by using metric scale. Measurement of dry weight of root and shoot was recorded after oven-drying the samples at 65℃ ± 2℃ for 72 hours. The dry was estimated by using electronic balance (Model BL2105, Starorious, Germany).

Measurement of photosynthetic rate

Photosynthetic rate (Pn) was measured using an Infrared Gas Analyzer (CID-340, Photosynthesis system, Bio-Science, USA). The measurements were taken in the morning (9:00–11:00 a.m.). At the time of analysis, average light intensity of growth chamber was ~800 µmol m−2 s−1, a relative humidity of 70%, an air temperature of ~28 °C. A CO2 level of ~380 µmol/mol was present in the air in one growth chamber. A CO2 level of ~550 µmol/mol was present in the air in second growth chamber. The IRGA was exposed to more than ~700 µmol m-2 s-1 of photosynthetic active radiation (PAR) before taking readings.

Estimation of soluble protein content

The soluble protein content was estimated by homogenized fresh leaf material (0.5 g) in 0.1 M phosphate buffer (pH 6.8 at 4 °C) with the help of pre-cooled mortar and pastle. The homogenate was transferred into 2 ml tubes and centrifuged at 5,000 × g at 4 °C for 10 min. Supernatant was transferred into new 2 ml tube and equal volume of chilled 10% TCA was added for protein precipitation. The whole mixture was centrifuged at 3,300 × g at 4 °C for 10 min, supernatant was discarded, and the remaining pellet was washed with cold acetone. The protein pellet was dissolved in 1.0 ml of 0.1 N NaOH. To 1.0 ml aliquot tube, 5.0 ml of 1×Bradford's reagent (Bio-Rad, USA) was added and vortexed. For optimal color development, tubes were kept in dark for 10 min and the absorbance was measured at 595 nm by microplate reader (Biotek Instruments Inc., USA). The soluble protein content was estimated with the help of a standard curve using Bovine Albumin Serum (Sigma-Aldrich, USA) as standard and expressed in mg g−1 FW (fresh weight)

III. RESULTS AND DISCUSSION

Plant biomass, plant height, leaf area were analyzed in both Rasi (V1) and CR Dhan (V2) varieties of rice under different treatments of N and CO2. Irrespective of treatments all the growth traits were higher in CR Dhan than Rasi.

Plant biomass

Plant biomass ranges between 0.257 to 0.366 g/plant in Rasi. In CR Dhan variety of rice, the range of biomass accumulation was 0.383 to 0.432 g/plant. Both the varieties of the rice responded differently to various treatments of N and elevated CO2. Compare to control, the biomass accumulation was significantly reduced at the treatment T2 and T4 in Rasi. There was no significant difference in the value of control and T3 in this variety. The percent reduction in the biomass accumulation was 16% and 25% at T2 and T4, respectively. In CR Dhan, there was no significant effect of treatments except T3 on the biomass accumulation. The T3 treatments resulted in an increase of 8.5% in the biomass accumulation when compare to control (Fig. 1).

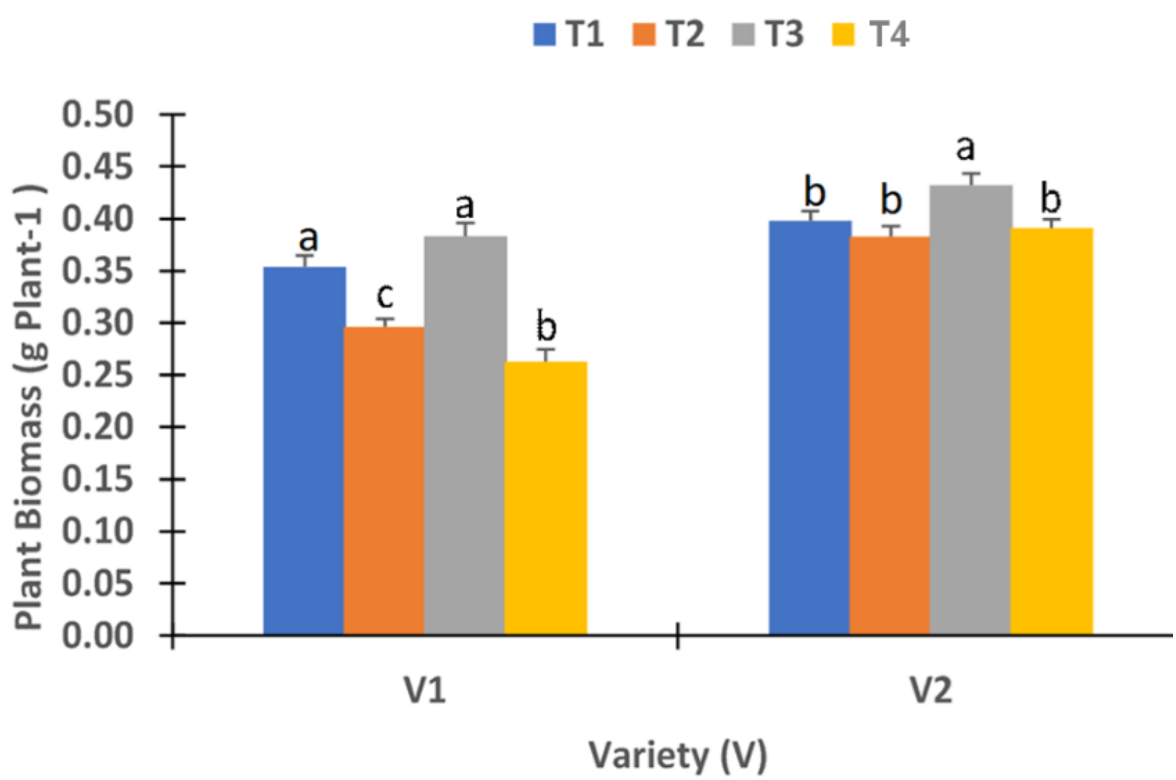

Figure 1. Plant biomass of Rasi (V1) and CR Dhan (V2) as affected by the treatments of nitrogen and CO_2 levels. Values are means of three independent replicates (n=3). Vertical bars show standard error. Values denoted by the similar letter was not significant statistically.

Plant Height

In terms of plant height, there was no significant effect of various treatment in CR Dhan, the plant height of Rasi was significantly affected by all the treatments when the comparison was made with the control. There was reduction in the plant height of Rasi at T2 (8.8%) and T4 (18.6%) when compare to control (T1). The T3 treatment increase the plant height of Rasi by 4.5% (Fig. 2).

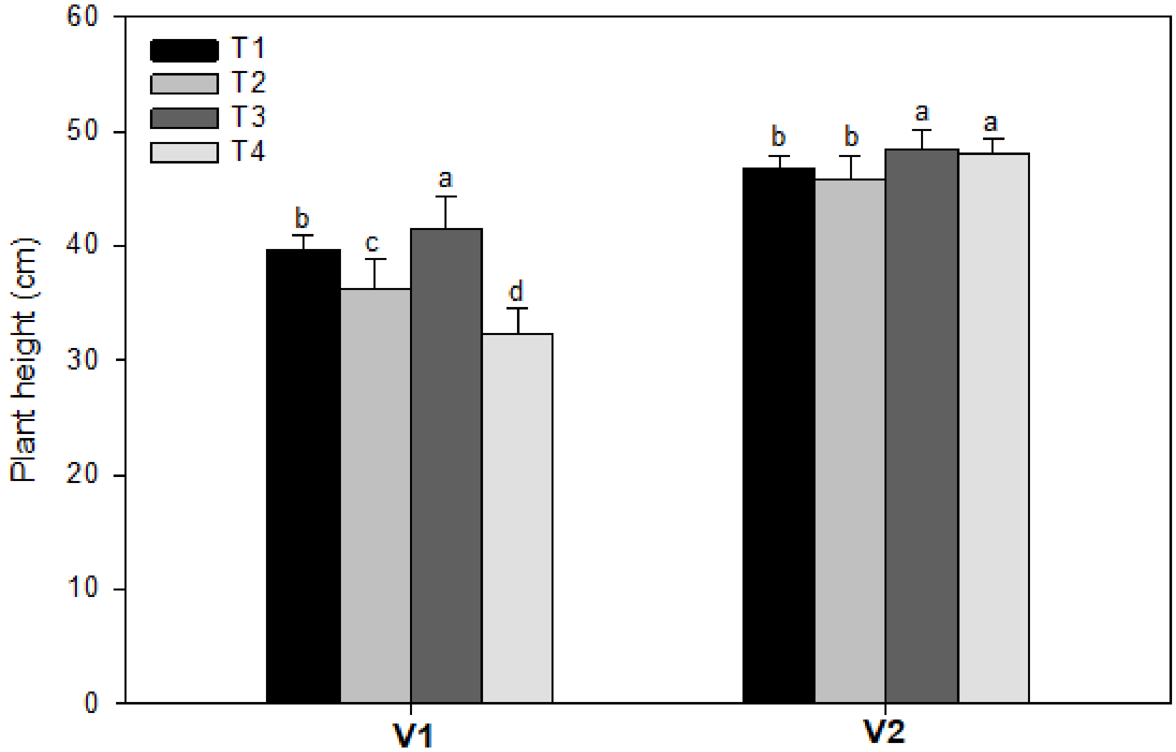

Figure 2. Plant height of Rasi (V1) and CR Dhan (V2) as affected by the treatments of nitrogen and CO_2 levels. Values are means of three independent replicates (n=3). Vertical bars show standard error. Values denoted by the similar letter was not significant statistically.

Leaf area

Leaf area of both the varieties as affected by various treatment is shown in figure 3. In Rasi, the leaf area was reduced by 14%, and 25% at treatment T2 and T4, respectively when compared with control (T1). At T3, there was increase (11%) in the leaf area of this variety. In CR Dhan, the leaf area was reduced by 4% at T2. However, there was increase in the leaf area at the treatment T3 and T4, when compared to control (Fig. 3).

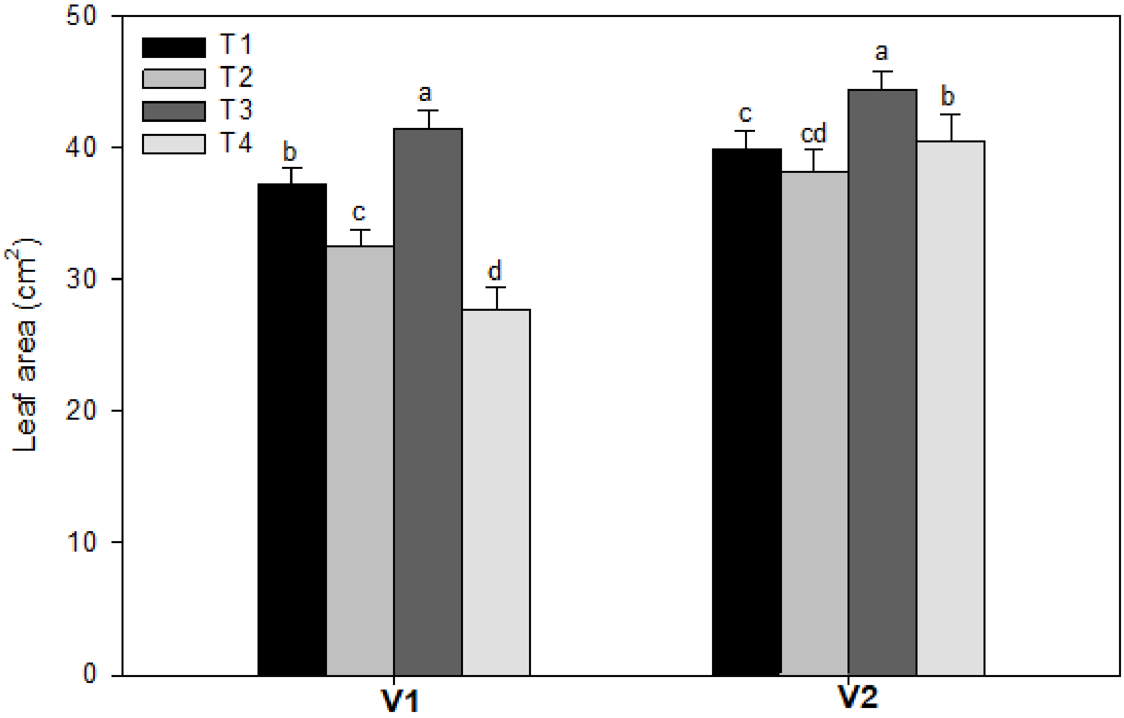

Figure 3. Leaf area of Rasi (V1) and CR Dhan (V2) as affected by the treatments of nitrogen and CO_2 levels. Values are means of three independent replicates (n=3). Vertical bars show standard error. Values denoted by the similar letter was not significant statistically.

Chlorophyll content

Irrespective of treatments, the value of SPAD was found lesser in Rasi when compared to CR Dhan. Irrespective of CO2 treatments when comparison was made between the normal and low level of nitrogen treatment, there was significant reduction in the value of SPAD in both the variety but the reduction in SPAD value was higher in Rasi than CR Dhan. Reduction in Rasi was 17%, whereas in CR Dhna only 3% reduction was found at T2 when compared to control. When comparison was made between T1 and T3 i.e., a comparison between ambient CO2 and elevated CO2 under optimum N condition, significantly higher chlorophyll content was found in both the varieties. when compared to T1, there was 26% reduction in the SPAD value of Rasi and 13% increase in SPAD value of CR Dhan at T4 (Fig. 4).

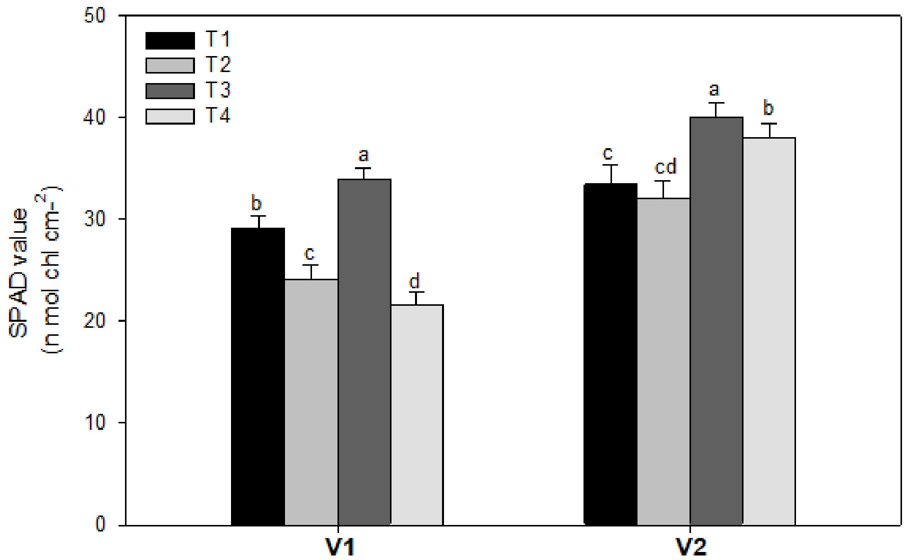

Figure 4. Chlorophyll content (SPAD values) of Rasi (V1) and CR Dhan (V2) as affected by the treatments of nitrogen and CO_2 levels. Values are means of three independent replicates (n=3). Vertical bars show standard error. Values denoted by the similar letter was not significant statistically.

Photosynthetic (Pn) Rate

Photosynthetic rate of both the varieties of rice was affected by different treatments of N and CO2 when compared with control. The Pn rate was found significantly higher in treatment T3 in both the varieties when compared with T1. In Rasi significant reduction was found at T4 (24%) when compared to T1. Whereas in case of variety CR Dhan the reduction was not significant at T4, compared to T1 (Fig. 5).

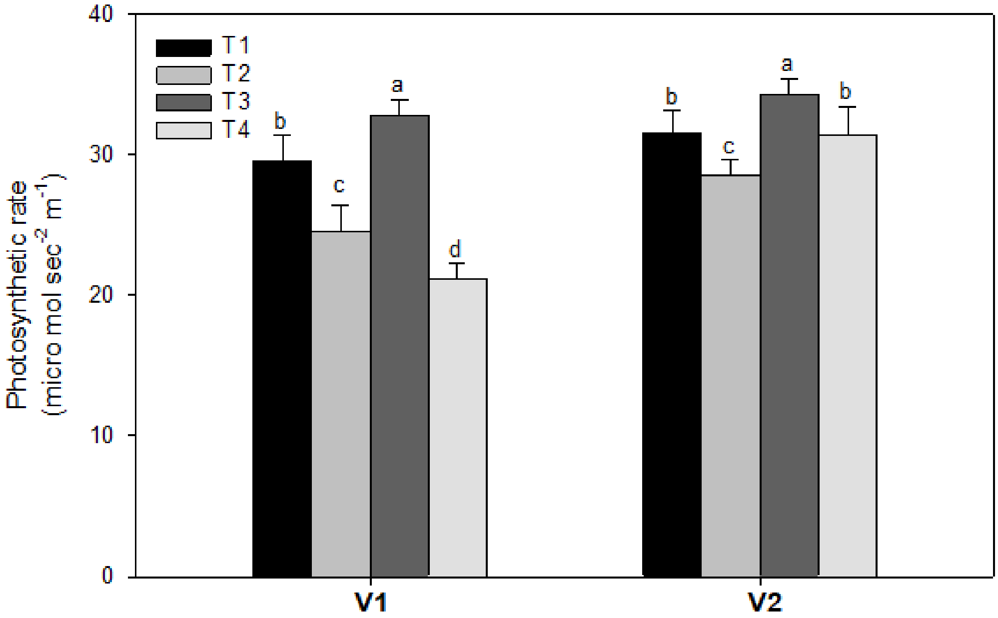

Figure 5. Photosynthetic rate of Rasi (V1) and CR Dhan (V2) as affected by the treatments of nitrogen and CO_2 levels. Values are means of three independent replicates (n=3). Vertical bars show standard error. Values denoted by the similar letter was not significant statistically.

For the growth and development of plants, it is utmost necessary that photosynthetic machinery adjusted to elevated CO_2 in view of the changing climatic conditions. An assessment of effect of elevated CO_2 was carried out in terms of growth and photosynthesis of rice varieties under low nitrogen conditions. Compared to nitrogen-efficient variety, nitrogen-inefficient variety of rice was more affected by the supply of low nitrogen under the elevated CO_2 conditions, showing that the requirement of nitrogen will be more under the elevated level of CO_2 in the environment. This increased requirement of nitrogen will create further severe environmental problem and financial burden. In view of current recommendations of the researchers to reduce the nitrogen load on the environment, additional need of the supply of nitrogen at the agricultural field to increase crop productivity is not favorable under the conditions of elevated CO_2 level. Under this situation a variety that does not require additional supply of nitrogen in the environment of elevated CO2 for maintaining its productivity is the need of the hour. CR Dhan variety of rice in our study might fulfil this criterion. However, additional details research is required for final selecting this variety.

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