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Impact of elevated carbon dioxide on the protective enzymes in brown planthopper (*Nilaparvata lugens*) and infested rice (*Oryza sativa*) plants

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ABSTRACT

The effect of elevated carbon dioxide (CO_2) on protein content, and catalase and peroxidase activity in rice (*Oryza sativa* L.) plant and brown planthopper (BPH) [*Nilaparvata lugens* (stal)] was studied in open top chamber (OTC) at elevated CO_2 (570±25 μl/l) compared to ambient CO_2 (400±25 μl/l). Uninfested rice plants under elevated CO_2 had lower protein content but increased activity of both catalase and peroxidase compared to ambient CO_2. The BPH infested rice plants had significantly lower protein content (15.89 g/l) and reduced catalase activity (0.78 μmoles of H_2O_2 decomposed/min/mg of protein) under both elevated and ambient CO_2 conditions compared to uninfested rice plant. However, the activity of peroxidase in infested rice plant was non-significantly increased at both elevated and ambient CO_2 compared to uninfested rice plants, indicating perhaps that it is an important enzyme in plant defence against BPH in addition to elevated CO_2. Further, lower protein content in rice plants under elevated CO_2 resulted in lower protein in BPH that fed on them. The activity of catalase and peroxidase in BPH was enhanced when they were fed rice plants under elevated CO_2 compared to ambient CO_2.

Key words: Catalase, Elevated CO_2, *Nilaparvata lugens*, Peroxidase, Protein, Rice

Rice (*Oryza sativa* L.) is indisputably the world's most important staple food that provides nutrition to more than half of the world's population (Khush 2004). It is attacked by several insect pests, which cause severe yield loss in one or the other region of India from nursery to harvest (Asghar *et al.* 2009). Among the sap sucking pests of rice, the planthoppers have become economically important all over the world (Magunmder *et al.* 2013). Brown planthopper (BPH) [*Nilaparvata lugens* (Stal)] (Hemiptera: Delphacidae) is one of the most destructive insect pests of rice. It causes direct damage by sucking phloem sap, causing leaf stippling, and indirect damage by transmitting viral diseases such as grassy stunt and rugged stunt (Reissig *et al.* 1986). The BPH is recognised for its ability to adapt in different ecological conditions and its regular impacts on rice yield (Lu *et al.* 2005).

Climate change is presently considered to be the most important factor influencing agricultural production. Greenhouse gases are contributing to the rise of temperature, among which carbon dioxide (CO_2) is the major gas. The atmospheric CO_2 concentration in the terrestrial troposphere was about 280 μ/l during pre industrial period and increased to 398 μl/l in 2014 (NOAA/ESRL 2014). These variations might have a direct impact on plant chemical constituents and metabolic enzymes of herbivorus insects.

The impact of elevated CO_2 on ecosystem is expectable to be enormous, especially on the growth and biochemical constituents of plants (Penuelas *et al.* 2002). Foliar amino acids and protein content in cotton plants lowered significantly under elevated CO_2 compared to ambient CO_2 (Wu *et al.* 2007). Changes in atmospheric CO_2 not only affect the plant quality but also herbivore performance (Awmack *et al.* 2004). Most herbivorous insects responded to elevated CO_2 by increasing developmental time, and reducing growth, survival rates, population densities and fitness, presumably because of the increased levels of carbohydrates in host plants (Yin *et al.* 2009). The carbon: nitrogen (C: N) ratio of the plant foliage generally increases when plants are grown under elevated CO_2 than in the ambient CO_2. As a result, insect larvae increase leaf consumption and their sucking rates under elevated CO_2 to compensate for lower nitrogen in plant foliage (Prasannakumar *et al.* 2012). Besides, size of plant canopy may also influence pest populations especially rice planthoppers through changes in micro-environment. Increase in C: N ratio of plants under elevated CO_2 leads to higher feeding rates of insects that may impinge upon level of host plant resistance against the pest. *N. lugens* fed on resistant rice varieties significantly enhanced glutathione S-transferase (GST) enzyme activity (Zhou *et al.* 2003).

Plants respond to insect infestation and/ or climate

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change by the development of the induced defence system by means of protective enzymes, viz. superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), peroxidase (POX), and glutathione reductase (GR) (Foyer *et al.* 1994). Morphological, physiological and biochemical changes in the form of accumulation of defensive compounds occur in the plants following herbivory. Reactive oxygen species (ROS) and antioxidative enzymes are recognised in response to herbivory and wounding (Orozco-Cardenas and Ryan 1999). According to Kogan and Paxton (1983), phenolic compounds were accumulated in plants following feeding of insects. Because of the difference in species and genotype of insects, there is a variation in the response of antioxidative systems to elevated CO_2 and temperature (Geetika 2003).

Elevated CO_2 is expected to alter plant physiological processes especially C: N ratio, which would subsequently influence the crop-pest interactions. It thus becomes imperative to investigate the effect of elevated CO_2 on both plants as well as insects at biochemical level. With this background, the present study was planned to estimate the protein content, catalase and peroxidase activity of rice plants subjected to elevated CO_2 and BPH infestation and also to estimate the protein content, catalase and peroxidase activity in BPH that fed on rice plants under ambient and elevated CO_2 .

MATERIALS AND METHODS

The study was undertaken at the ICAR-Indian Agricultural Research Institute (IARI), New Delhi (28°38´N latitude, 77°09´E longitude, 228.61 m altitude) during July-October 2016. Seeds of Pusa Basmati 1121, susceptible rice cultivar to *Nilaparvata lugens*, were sown in a nursery (measuring 3×3 sq m). One month old rice seedlings were transplanted in 20 experimental pots (15×9×9 cm^3) under natural condition. Two rice seedlings were transplanted per pot and allowed to establish for 10 days. Ten potted plants each of 10-day old were transferred to open top chamber (OTC) with elevated CO_2 (570±25) µl/l and ambient CO_2 (400±25) µl/l level. No insecticide was applied to the plants throughout the study.

Brown planthopper collected from rice fields of IARI were reared and maintained under glasshouse in wire-mesh wooden cages (60×45×45 cm). Out of 10 pots each under elevated and ambient CO_2, five pairs (1:1 sex ratio) of BPH adults were released in five pots apiece, after 10 days of exposure of potted plants to CO_2 in OTCs. Remaining pots were maintained as control without BPH under both the OTCs. The biochemical assay was carried out on BPH infested and uninfested rice plants after one month of exposure of plants to CO_2 in OTCs. Likewise, BPH nymph were collected and analysed for biochemical changes, after one month of release of BPH on rice seedlings at both elevated and ambient CO_2.

BPH infested and uninfested plant samples (tillers) were collected from the experimental potted plants maintained at elevated and ambient CO_2 and were stored at -80°C for further analysis. The samples were homogenised by hand held motorised homogeniser with addition of 150 µl homogenization buffer (potassium phosphate, 50 mM; pH 7). The homogenate was centrifuged at 12,000 rpm for 30 min at 4°C, and the supernatant was used for energy assay. Protein content was determined using Bradford reagent (Bradford 1976), with bovine serum albumin as the standard. The activity of catalase and peroxidase was analyzed according to the protocol described by Sinha *et al.* (1972) for catalase and Reddy *et al.* (1995) for peroxidase with suitable modifications. Enzyme activity of catalase and peroxidase was computed relative to total protein content. Each test was replicated at least three times. The biochemical assay was carried in infested plants to assess whether the host plant defensive system varied with the injury by the BPH nymphs.

After one month of release of BPH on rice plants in OTCs, five third instar nymphs were collected in 2 ml micro centrifuge tubes in three replicates each from rice plants both under elevated and ambient CO_2 and stored at -80°C for further analysis. The frozen samples were ground with homogenizer after adding homogenization buffer (Potassium phosphate 50 mM, pH 7). The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was analyzed. Protein content and the activity of protective enzymes were estimated as described above. Catalase activity (µmoles of H_2O_2 decomposed/min/mg protein) and peroxidase activity (Δ in optical density (OD)/min/mg protein) was calculated relative to total protein content. Each test was replicated at least three times.

The mean protein content, and catalase and peroxidase activity in BPH infested and uninfested rice plant subjected to elevated and ambient CO_2 were analysed through two factorial one way analysis of variance (ANOVA) in Microsoft Excel. Mean differences were compared by least significant difference (LSD) test when significant F-values were obtained ($P<0.05$). The mean protein content and enzyme activities in *N. lugens* nymphs were compared by paired t-test ($P<0.05$).

RESULTS AND DISCUSSION

The results of the study on effect of elevated CO_2 and BPH infestation on rice plants revealed that protein content differed significantly across CO_2 levels (P=0.0002, Fig $1A_1$) and infestation levels (P=0.001, Fig $1A_2$). Rice plants had higher protein content (20.37 g/l) under ambient CO_2 as compared to elevated CO_2 (15.22 g/l) across infestation levels (Fig $1A_1$). Similarly, uninfested rice plants had higher protein content (19.71 g/l) as compared to infested rice plant (15.89 g/l) across CO_2 levels (Fig $1A_2$). The elevated CO_2 and BPH infestation thus reduced the protein content in rice plants. However, interaction effect between the BPH infestation and CO_2 levels in regard to protein content in plants was not observed.

Catalase (P=0.0001) and peroxidase activity (P=0.00007) in rice plants differed significantly between CO_2 levels across BPH infestation levels, while enzymes activity did not differ

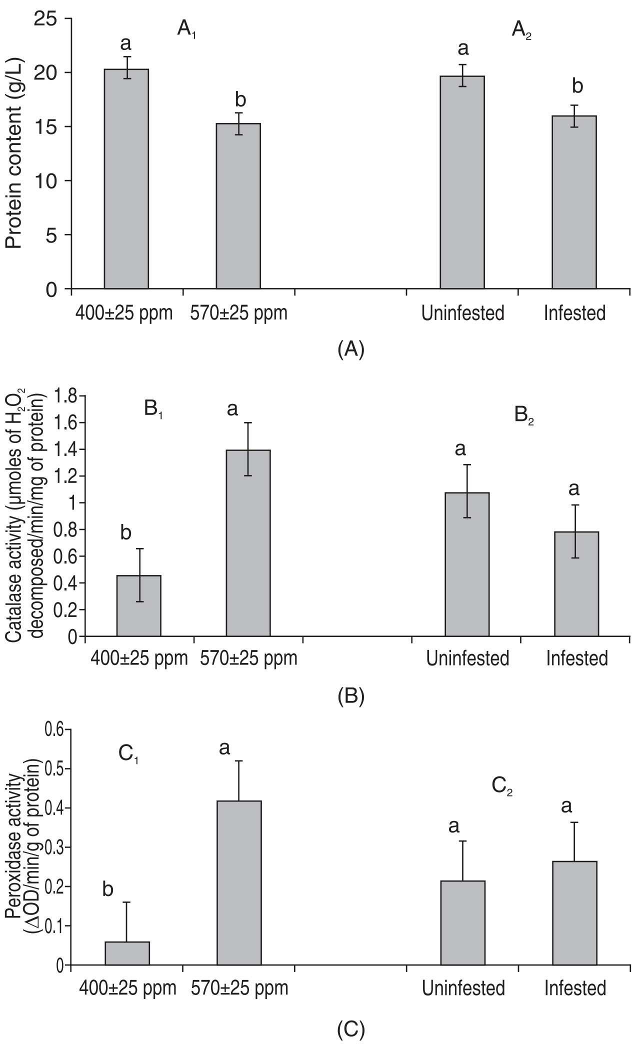

Fig 1 Protein content and protective enzymes activity in BPH infested and uninfested rice plants at elevated and ambient CO_2 condition. (A) Protein content, i.e. g/l (mean±SE), (B) Catalase activity, i.e. µmoles of H_2O_2 decomposed/min/mg of protein (mean±SE) and (C) Peroxidase activity, i.e. ΔOD/min/g of protein (mean±SE). Different lowercase letters indicate significant difference between treatments by least significant difference (LSD) test. A_1: Comparison between CO_2 levels (LSD: 1.935), A_2: Comparison between BPH infestation levels (LSD: 1.935), B_1: Comparison between CO_2 levels (LSD: 0.32), B_2: Comparison between BPH infestation levels (Non-significant), C_1: Comparison between CO_2 levels (LSD: 0.111), C_2: Comparison between BPH infestation levels (Non-significant).

significantly between infestation levels across CO_2 levels. The interaction effect between the BPH infestation and CO_2 levels in regard to catalase and peroxidase activity in plants was not observed.

Rice plants had significantly higher catalase activity (1.39 µmoles of H_2O_2 decomposed/min/mg of protein, Fig $1B_1$) under elevated CO_2 compared to ambient CO_2 (0.45 µmoles of H_2O_2 decomposed/min/mg of protein) across infestation levels. The BPH fed rice plants had reduced catalase activity (0.78 µmoles of H_2O_2 decomposed/min/mg of protein, Fig. $1B_2$) compared to uninfested plants (1.07 µmoles of H_2O_2 decomposed/min/mg of protein) across the CO_2 levels thereby impairing the plant defence against elevated CO_2.

Similarly, rice plants had higher peroxidase activity (0.41 ΔOD/min/g of protein, Fig. $1C_1$) under elevated CO_2 compared to ambient CO_2 (0.06 ΔOD/min/g of protein), across BPH infestation levels. The peroxidase activity increased non-significantly in infested rice plants (0.264 ΔOD/min/g of protein, Fig $1C_2$) compared to uninfested rice plant (0.215 ΔOD/min/g of protein) across CO_2 levels. BPH feeding thus increased peroxidase activity in rice plants. Catalase activity decreased, whereas peroxidase activity increased in response to BPH infestation indicating perhaps peroxidase could be involved in plant defence against BPH besides stress posed due to elevated CO_2.

The protein content in BPH nymphs (t=3.875, df=2, P=0.03; Fig 2A) under elevated CO_2 was significantly lower than ambient CO_2. The BPH that fed on rice plants under elevated CO_2 having lower protein content resulted in lower protein in insects. However, the activity of catalase (t=9.066, df=2, P=0.012; Fig 2B) and peroxidase in BPH nymphs (t=5.937, df=2, P=0.027; Fig 2C) was significantly higher under elevated CO_2 compared to ambient CO_2.

In most of the situation, rice plant responded to the elevated CO_2 by increasing the growth and development, and decreasing the foliar nitrogen and total amino acids, primarily because of build up of non-structural carbohydrates (Wu *et al.* 2007). In the present study, we observed reduced protein content under elevated CO_2 but the activity of catalase and peroxidase was enhanced in rice plant under elevated CO_2 that was consistent with earlier reports of lower protein content in rice at elevated CO_2 (Zeng *et al.* 2012). However, earlier studies reported reduced catalase activity in spruce and tobacco (Havir and McHale 1989), but there was no effect of elevated CO_2 on catalase activity in oak, pine and orange leaves (Schwanz *et al.* 1996). Under elevated condition, the amount of sucrose, glucose, free amino acids, soluble proteins, total non-structural carbohydrates, free amino acids, and nitrogen had increased (Chen *et al.* 2004). Although the effect of elevated CO_2 on plant and insect biochemical constituents often change with time, this has not been taken into account in most studies attempting to characterize the effect of CO_2 enrichment on antioxidant activity. Furthermore, there may be significant intra- and inter-specific changes in response of plant antioxidative enzymes to elevated CO_2 (Badiani *et al.* 1993).

It has been observed earlier that, there was a quantitative variation in biochemical constituents of the most infested plants in terms of increase in levels of proteins, phenols, carbohydrates and enzymes such as peroxidase and catalase activities (Willaims *et al.* 1998). Our study showed that protein content and catalase activity were reduced, while peroxidase activity increased in BPH infested rice plants compared to uninfested rice plants. This has also been reported due to Russian wheat aphid infestation (Mohase and Van der Westhuizen 2002). In

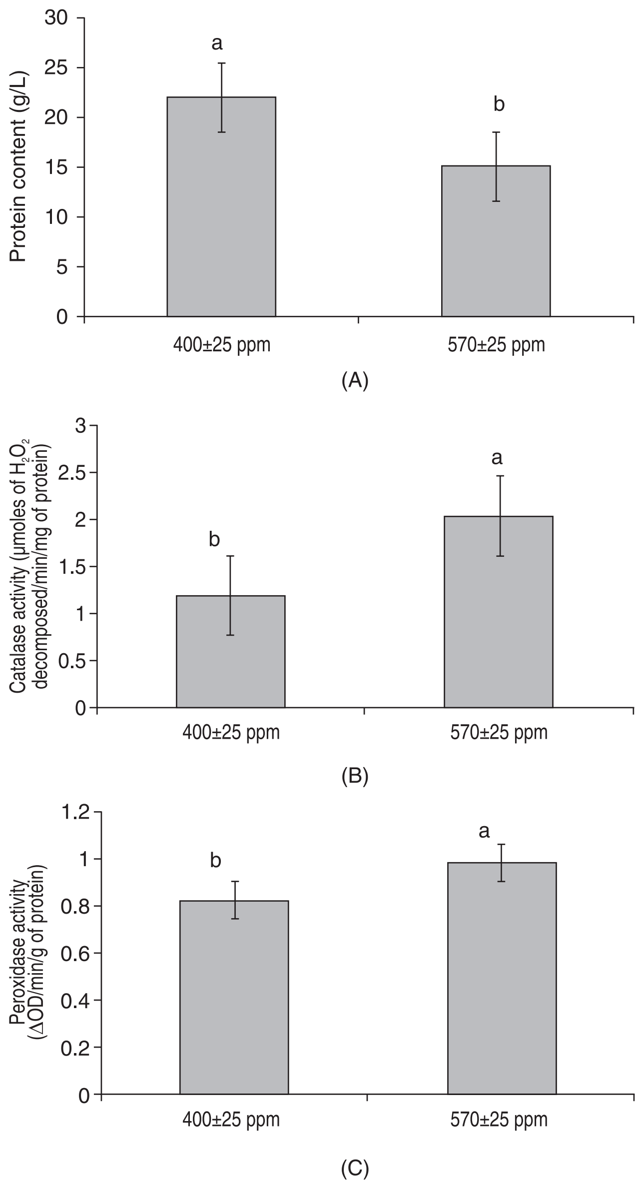

Fig 2 Protein content and protective enzymes activity in BPH nymphs at elevated and ambient CO_2 condition. (A) Protein content, i.e. g/l (mean±SE), (B) catalase activity, i.e. µmoles of H_2O_2 decomposed/min/mg of protein (mean±SE) and (C) peroxidase activity, i.e. ΔOD/min/g of protein (mean±SE). Different lowercase letters indicate significant difference between treatments by the paired T test (*P*<0.05).

contrast, the increased catalase activity had been reported in yellow stem borer and leaf roller infested rice plants (Usha Rani and Jyothsna 2009) and also in *Helicoverpa zea* infested soybean plants (Bi and Felton 1995), probably because plant response depends on the type of insect feeding. The homopterans feed from the contents of vascular tissue by inserting a stylet between the adjacent cells, thus limiting cell damage and lowering the induced response. The increase in peroxidase activity in BPH damaged plants could be attributed to the fact that it is the key enzyme which participates in several plant cell wall building processes (Chittoor *et al.* 1999).

Insects responded to reduced nutrient content by lowering their behaviour performance and to increased amount of carbohydrates of plants under elevated condition by increasing development time and by reducing growth and survival rates, population densities and fitness (Yin *et al.* 2009). Our results showed that elevated CO_2 reduced the protein content but the activity of protective enzymes, viz. catalase and peroxidase was enhanced in BPH which is was consistent with the earlier reports of enhanced catalase and superoxide dismutase activity in BPH under adverse environmental condition or host plants as protective enzymes provided most important defence to the herbivory (Wu *et al.* 2011). Activities of catalase and cholinesterase of cotton bollworm was significantly affected under elevated condition (Wu *et al.* 2010). A strong correlation occurred between enhanced activities of protective enzymes catalase and peroxidase, and insect adaptation to harsh ecological condition rather than detoxification and digestive enzymes (Zeng *et al.* 2012).

The present study on impact of elevated CO_2 on rice plants and BPH nymphs concludes that elevated CO_2 decreased protein content and increased activity of protective enzymes in both rice plant and insects. This increase in activity of protective enzymes, adapts the insects and plants to accustom in adverse climatic conditions. BPH infestation on rice plants reduced the protein content and catalase activity in rice plants but increased peroxidase activity indicating that it is an important enzyme involved in plant defence against insects.

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