Silicon (2023) 15:5811–5820
https://doi.org/10.1007/s12633-023-02480-w

RESEARCH

Effect of Silicon Application to the Rice Plants on Feeding Behaviour of the Brown Planthopper, *Nilaparvata lugens* (Stål) Under Elevated CO_2

Prabhulinga Tenguri[1,2] · Subhash Chander[3] · Ranjith Kumar Ellur[1,4] · Yogesh Yele[1,5] · Arya Pattathanam Sundaran[1] · Madhu Tadagavadi Nagaraju[6] · Sabtharishi Subramanian[1] · Sachin Suresh Suroshe[1]

Received: 2 March 2023 / Accepted: 18 April 2023 / Published online: 27 April 2023

Abstract

Silicon (Si) is known to enhance plant resistance in rice and many other Poaceae plants by priming chemical defence, physiological, and mechanical barriers. However, the impact of soil application of Si on the feeding behaviour of rice Brown Planthopper (BPH) under climate change conditions remains unknown. This study aims to investigate the effects of Si on BPH feeding behaviour under elevated CO_2 levels (570 ± 25 ppm) and elevated temperature ($\simeq 3$ °C higher than ambient) in open-top chambers (OTCs) during the Kharif season of 2019 and 2020. The results of the study showed that honeydew excretion by BPH was reduced by Si amendment, indicating reduced feeding by the pest under both ambient and elevated CO_2 and temperature conditions. The deposition of callose in the sieve tube of rice leaf sheath was identified as one of the main reasons, along with silicification, for reduced feeding in response to BPH infestation. Both Si-amended and non-amended plants showed a positive response to BPH infestation by depositing callose. In Si-amended plants, BPH infestation induced higher expression of the callose synthase-encoding gene (*OsGSL1*) and lower expression of the gene encoding callose hydrolase (*Gns5*) compared to non-amended plants. The dynamic expression of these two genes indicated higher callose deposition in Si-amended plants. The benefits of Si, which include reduced feeding by BPH through callose deposition, mechanical barrier *via* silicification, and positive alteration of photosynthesis-related parameters, suggest its potential as an alternative strategy for BPH management under climate change conditions.

Keywords Rice · Silicon · BPH · Honeydew · Silica cells · *OsGSL1* gene · *Gns5* gene

---

✉ Subhash Chander
schanderthakur@gmail.com

Prabhulinga Tenguri
prabhut07@gmail.com

Ranjith Kumar Ellur
ranjithellur@gmail.com

Yogesh Yele
yogeshyele13@gmail.com

Arya Pattathanam Sundaran
arya20811iari@gmail.com

Madhu Tadagavadi Nagaraju
madhuentomology@gmail.com

Sabtharishi Subramanian
entosubra@yahoo.co.in

Sachin Suresh Suroshe
sachinsuroshe@gmail.com

[1] Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi 10012, India

[2] ICAR-Central Institute for Cotton Research (CICR), Nagpur, Maharashtra 440010, India

[3] ICAR-National Centre for Integrated Pest Management (NCIPM), New Delhi 110012, India

[4] Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi 110012, India

[5] ICAR-National Institute of Biotic Stress Management (NIBSM), Raipur, Chhattisgarh 493225, India

[6] ICAR- Central Plantation Crops Research Institute (CPCRI), RS, Vittal, 574243 Dakshina Kannada, India

1 Introduction

Rice, *Oryza sativa* L. (Poaceae), is the world's most important cereal crop, widely cultivated in tropical and subtropical countries. It grows widely in India under a variety of meteorological conditions, from 8 to 35° N latitudes and from sea level to 3000 m. Rice is the staple food for more than half of the world's population. However, biotic and abiotic stresses have an impact on its production. Insect pests are the most significant biotic constraint to rice production. Each year, biotic factors cause loss of more than 52% of the total global rice crop, with insect pests accounting for nearly 21% of this loss [1]. More than 100 insect species attack rice crops, with roughly 20 of them being major pests, and one of them is the Brown Planthopper (BPH), *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae) [2]. BPH nymphs and adults cause damage both directly and indirectly by feeding on phloem sap and transmitting virus diseases such as rice grassy stunt, and ragged stunt [3]. BPH infestation has hampered rice production in Asia in recent years [4-6]. If 100–200 first-instar BPH nymphs attacked the plant 25 days after rice seedling transplanting, the yield would be reduced by 40–70% [7]. Furthermore, new research shows that rice production, or the agricultural production system, is very vulnerable to climate change. The rise in CO_2 and temperature may have an immediate impact on food grain production as well as an indirect effect on crop pests [8]. Climate change has a significant impact on the distribution and abundance of insects. Populations of *N. lugens* [9], *Sitobion avenae* [10], and *Macrosiphum euphorbiae* [11] increased with increasing CO_2 compared to ambient CO_2. The combined effects of high temperatures and CO_2 changed plant phenology and pest biology, exacerbating the damage caused by *Rhopalosiphum maidis* and *M. euphorbiae* [12]. Under elevated CO_2 and temperature conditions, the developmental period of BPH nymphs and the longevity of brachypterous females were shortened [13]. Additionally, increased CO_2 affected the biochemical composition of plants as well as the dynamics of defensive antioxidant enzymes in response to herbivory [14]. In all cases, chemical pesticides were used to regulate BPH; however, indiscriminate use of these insecticides has severe harmful impacts on the environment and human health. It is critical to manage the BPH population through the development of resistant varieties by identifying the resistance source from genotypes [6, 15], ecological engineering [16], and other such effective and ecologically sound alternative methods. One of these prospective solutions for BPH management is Si amendment or the external application of a plant-available form of Si. According to some recent studies, Si influenced plant defence both directly and indirectly, directly by priming chemical defence reactions in plants [17], by decreasing the digestion efficiency in herbivores [18, 19], by intensifying physiological and mechanical barriers caused by amorphous silica deposition in plant tissues [20], and indirectly by natural enemy attraction through herbivore induced plant volatiles [21, 22]. It also reduced honeydew excretion and feeding by depositing callose in phloem sieve tubes [23]. However, it is unclear whether Si plays a role in the reduction of BPH feeding under climate change condition. Thus, in the context of climate change, the current study investigated experimentally how BPH damage to the rice plant can be reduced using Si amendment.

2 Materials and Methods

2.1 Stock Culture of Test Insect

The BPH population was collected from rice fields of ICAR-Indian Agricultural Research Institute (IARI), New Delhi (28^{o}38'N latitude, 77^{o}09'E longitude, 228.61 m altitude), India. The population was reared on TN1 and *Pusa Basmati* 1121 rice varieties in the glasshouse with optimum rearing conditions of $27+2^0$ C and 75% ± 5% relative humidity and 14 h light/10 h dark photoperiod according to Heinrichs et al. [24]. Further, all the experiments were carried out by using the established population.

2.2 Preparation of Soil and Seedling for Transplanting

Si in the form of calcium silicate, $CaSiO_3$ ($\geq$87% SiO_2 and 12–22% CaO) was used to treat the soil in 3 different concentrations viz., 0 g, 0.16 and 0.32 g Si per Kg soil. The 21 days old *Pusa Basmati* 1121 seedlings were transplanted in pots (25 × 22 cm) filled with Si treated soil after root treatment of 0.2% carbendazim. After transplanting, the pots were kept for seedling stabilization before shifting them to the Open Top Chamber (OTC) facility.

2.3 Treatments in Open Top Chamber (OTC) Facility

To evaluate the combined effect of elevated CO_2 and Si amendments on rice plants and BPH, rice plants were exposed to different levels of CO_2 and Si amendments with and without inoculation of BPH (denoted as + BPH and – BPH) to ascertain the direct and indirect impact of these treatments on BPH feeding. This experiment was conducted under OTCs, four OTCs were used in the study; two OTCs for elevated CO_2 studies and two for ambient CO_2 effect studies. In total there were 12 treatments with 5 replications.

Treatments:

T1: Elevated $CO_2 + 0$ g Si + BPH
T2: Elevated $CO_2 + 0.16$ g Si + BPH
T3: Elevated $CO_2 + 0.32$ g Si + BPH
T4: Elevated $CO_2 + 0$ g Si – BPH
T5: Elevated $CO_2 + 0.16$ g Si – BPH
T6: Elevated $CO_2 + 0.32$ g Si – BPH
T7: Ambient $CO_2 + 0$ g Si + BPH
T8: Ambient $CO_2 + 0.16$ g Si + BPH
T9: Ambient $CO_2 + 0.32$ g Si + BPH
T10: Ambient $CO_2 + 0$ g Si – BPH
T11: Ambient $CO_2 + 0.16$ g Si – BPH
T12: Ambient $CO_2 + 0.32$ g Si – BPH

The climatic conditions prevailed during the experiments was as follows, in 2019, the average minimum temperature, maximum temperature, and CO_2 concentration were 16.7 °C, 31.2 °C, and 408.75 ppm, while in 2020, they were 18.4 °C, 30.6 °C, and 411.65 ppm, respectively.

2.4 Honeydew Excretion Analysis

Honeydew excretion by BPH is correlated with their feeding behaviour. To assess the impact of various treatments on BPH feeding behavior, we employed a parafilm sachet method [25] to quantify honeydew excretion. We placed a single pair of recently emerged female insects within a 4×4 cm parafilm sachet, with the sachet opening attached to the stem of 60-day-old rice seedlings. The insects were allowed to feed freely on the rice leaf sheath via the sachet opening [23]. At 24, 48, 72, and 96 h post-infestation (hpi), we removed the insects and weighed the sachets immediately. The net weight of honeydew was calculated by subtracting the weight of the empty sachet from the final sachet weight. We repeated the experiment five times.

2.5 Expression of Callose Synthase (*OsGSL1*) and the Callose Hydrolase β-1,3-glucanase (*Gns5*) Genes upon BPH Infestation

The callose is a polysaccharide and its synthesis or deposition is coordinated through the expression of the genes encoding callose synthase (OsGSL1) and hydrolase (Gns5). Induction of callose deposition and metabolism is correlated with reduced feeding by BPH. Thus the established correlation of mRNA levels of these with callose deposition was used to quantify the callose in Si-treated plants by using qRT-PCR. We quantified the relative expression levels of *OsGSL1* (Accession number AP001389) and *Gns5* (Accession number U72251) genes in Si amended plants that were infested at different time intervals under elevated and ambient CO_2.

The leaf-sheath samples were collected at different time point's viz., 0, 6, 12, 24, 48 hpi and stored immediately at – 80 °C. The total RNA was extracted from each sample by using Macherey-Nagel DNA, RNA % protein purification kit (Germany) following the manufacturer's instructions. For each total RNA sample, 1 μg of RNA was reverse-transcribed to cDNA by the Affinity Script QPCR cDNA synthesis kit (USA). Two reference genes *Actin1* and *18 S* were used for normalization. The *OSGSL1* and *Gns5* genes were synthesized using the methods described by Hao et al. [23]. Similarly, the reference genes were synthesized based on the protocols outlined in the studies by Du et al. [26] and Kim et al. [27]. The details of the primers are given in the S1.

To measure the expression levels of *GNS5* and *OSGSL1*, we used the QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems, USA) and followed the method described by Yang et al. [28]. We performed the qPCR in triplicate and calculated the average result for each of the three biological replicates. We used the $2-\Delta\Delta$Ct method [29] to determine the relative expression levels of the two genes.

2.6 Microscopic Observation of Silica Cells

Scanning electron microscopy (SEM) was used to examine the morphology of silica cells on the leaf surface (Tescan-Vega3, France). A fresh leaf sheath about 0.3–0.5 cm in length was collected and cleaned with tissue paper. The leaf segment was fixed for 2–3 h at 4 °C with 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4), and then post-fixed for 30 min with 1% (w/v) osmium tetroxide in the phosphate buffer solution. Following that, the leaf sample was dehydrated in a graded ethanol series [50, 70, 80, 90, and 100% (v/v)], dried with liquid CO_2, and coated with palladium metal before being put into the SEM. SEM images (at 300 magnification) were taken to demonstrate the differences in silica cells of rice leaves between treatments.

2.7 Histological Observation of Callose Deposition

Yang et al. [27] method was followed to localize callose deposition in Si treated and untreated rice plants upon BPH infestation. Two pairs of newly emerged adult females were confined to a parafilm sachet fastened to the leaf sheath of 60 days old plant. Leaf-sheath samples were collected at 24, 48, 72, and 96 hpi from infested ones and those without BPH infestation. To determine the number of sieve plates with callose deposition, we prepared cross-sections of leaf sheath samples and examined them using a fluorescence microscope (Zeiss Axio imager.M2m, Germany) following the method described by Yang et al. [27]. We identified callosic plates as those exhibiting bright blue fluorescence [30] and recorded the number of callosic sieve plates from 30 cross-sections per plant, with 5 plants observed for each treatment. Photographs were obtained by using a Leica SP5 laser scanning confocal microscope.

2.8 Measurement of Photosynthesis and Related Parameters

Photosynthesis related parameters viz., photosynthetic rate (P), transpiration rate (E), stomatal conductance (gs), water use efficiency (WUE), and sub-stomatal CO_2 concentration (Ci) were measured from the 45 days old rice leaf sheath using an infrared gas analyzer (LI6400*XT* portable photosynthesis system, LI-COR, Inc, Nebraska, USA). All the observations were recorded between 9 and 11 AM in 30 replicates for each of the treatments.

2.9 Statistical Analysis

The experimental data was analysed using statistical analysis of variance (ANOVA) for completely randomized design (CRD) with the assistance of R software [31]. Prior to analysis, all data were checked for normality, and if necessary, appropriate arcsine and square root transformation methods were used. Treatment means were distinguished using the least significant difference test (LSD) at $P = 0.05$ [32], and GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA) was used to create graphs.

3 Results

3.1 Honeydew Excretion by BPH Under Elevated and Ambient CO_2

Time-series observation on honeydew excretion revealed a significant effect of Si amendment on it. Honeydew excretion was found to increase as the BPH feeding duration increased until 96hpi and peaked at 72hpi ($p < 0.01$) (Fig. 1). A similar trend was evident with all three doses of Si treatment under both elevated and ambient CO_2 conditions (Fig. 1).

3.2 Gene Expression of Callose Synthase and Hydrolase

The relative expression of *OsGSL1* and *Gns5* was measured using the qRT-PCR technique. Under elevated CO_2, the *OsGSL1* gene was up regulated in increasing order with the BPH feeding duration till 12 hpi. However, later on, the expression showed a declining trend in all three doses of Si treatment. It was upregulated by 2.11, 3.86, 5.74 times at 6 hpi and 3.23, 7.42, 9.49 folds, respectively at 12 hpi respectively in 0, 0.16, and 0.32 g Si treatments. However, among different Si treatments, 0.32 g Si treatment showed 2.74 times and 0.16 g Si treatment showed 1.48 times expression of *OsGSL1* gene compared to 0 g Si treatment at 6 hpi and it was 2.93 times with 0.32 g Si and 1.27 times with 0.16 g Si at 12 hpi. Thereafter, the expression was less upregulated (Fig. 2). Under ambient CO_2, a similar trend of gene expression between different doses of Si and BPH feeding duration was observed.

The expression of the *Gns5* gene was upregulated less during the early hours of feeding duration. The expression was upregulated more after 12 hpi and its expression pattern was almost opposite to the *OsGSL1* gene expression. It was upregulated by 4.3, 10.04, 11.39 folds, respectively in 0, 0.16, and 0.32 g Si treatments at 12 hpi. Unlike at 12 hpi, the gene was more expressed in 0 g compared to 0.16 g Si and 0.32 g Si by 1.56 & 2.10 and 0.87& 2.75 times, respectively at 24 and 48 hpi (Fig. 2). These trends of results under elevated CO_2 are almost similar to those under ambient CO_2 (Fig. 2), indicating that the *Gns5* gene expression was influenced by the Si amendment but not by the levels of CO_2.

3.3 Histological Observation on Callose Deposition

The callose deposition in the sieve tube of rice leaf sheath in response to BPH feeding was recorded through histological observations. The callose deposited on the sieve tube was observed to be thickened and emitted strong fluorescence through the fluorescent microscope in Si amended plants. Also, the feeding duration of BPH influenced the number of callose on sieve plates. The number of callose deposited on the sieve plate increased from 2.67 to 11 in

Fig. 1 Honeydew excretion by BPH from the rice plant treated with 0, 0.16 and 0.32 g Si per Kg soil at different time point of BPH infestation (24, 48, 72 and 96 hpi) under elevated and ambient CO_2.

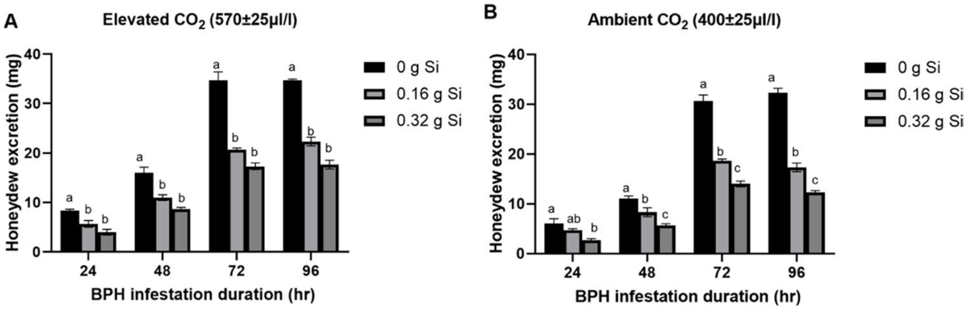
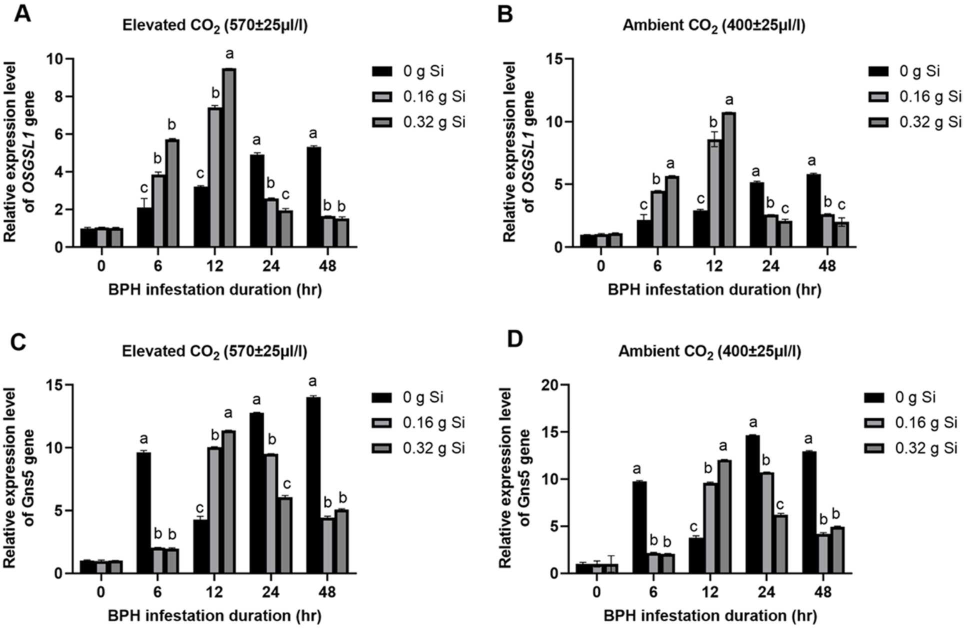


Fig. 2 Relative expression levels of callose synthase (*OSGSL1*) and hydrolase (*Gns5*) genes in rice plant treated with 0, 0.16 and 0.32 g Si per Kg soil at different time point of BPH infestation (0, 6, 12, 24 and 48 hpi) under elevated CO_2 with Silicon amendment



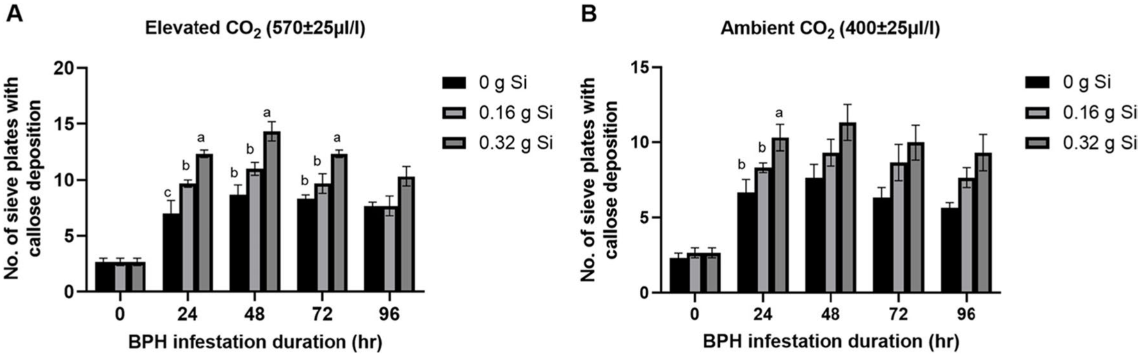
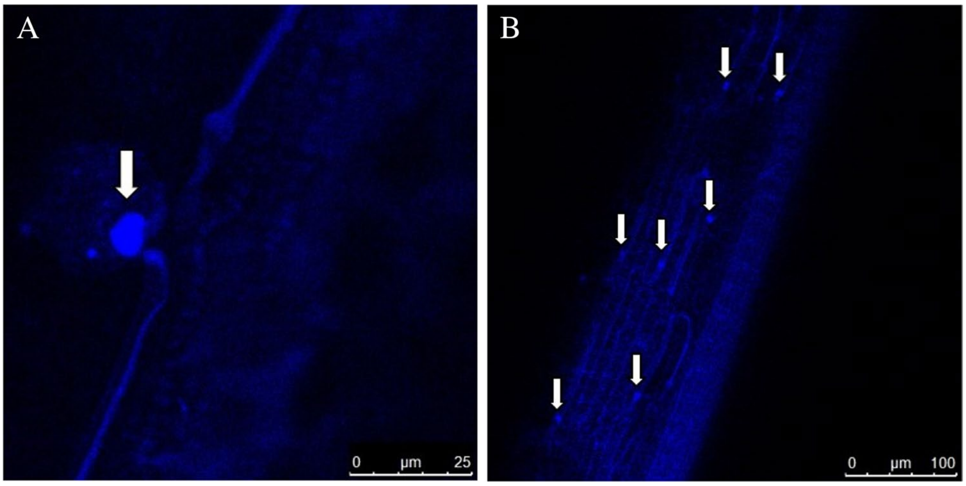


Fig. 3 Callose deposition in rice leaf sheath in response to silicon amendment with 0, 0.16 and 0.32 g Si per Kg soil at different time point of BPH infestation (0, 24, 48, 72 and 96 hpi) under elevated and ambient CO_2 conditions

Fig. 4 Callose deposition in rice leaf sheath tissues obtained with a confocal microscope. Bright blue fluorescence indicates the induced callose in the Si amended plant in response to BPH feeding. The white arrow marks on **A** indicates the single callose (scale bar = 25 µm) and white arrow marks on image **B** indicates the presence of number of callose in the sieve tube of the rice leaf sheath (scale bar = 100 µm)



0.32 g Si treated plants compared to 9.6 and 8.3 in 0.16 g and 0 g Si treated plants, respectively (Fig. 3). The callose deposition increased from 0 to 48 hpi and later it showed a declining trend both under elevated and ambient CO_2. However, the number of callose was observed to be considerably more under elevated CO_2 plants than ambient CO_2 plants (Fig. 4). It indicated Si amendment significantly increased the callose deposition in rice plants during the early period of BPH feeding than later as a means of protection against the biotic stress. *Silicification*: Morphological observation of leaf sheath by scanning electron microscope showed the distribution of silica bodies (Fig. 5). The density and size of the silica bodies increased with increase in Si application.

3.4 Effect of Si Amendment on Photosynthesis-Related Parameters with BPH Infestation Under Elevated and Ambient CO_2

Photosynthesis-related parameters of all the treatment plants were computed after measurement by IRGA and the analysis revealed significant differences with Si amendment and BPH infestation under elevated and ambient CO_2. Under elevated CO_2 with BPH infestation, photosynthetic rate and WUE significantly increased with Si amendment. It increased by 24.26% and 6.88%, respectively in 0.32 g Si treatment compared to 0 g Si and in 0.16 g Si treatment, photosynthetic rate and WUE increased by 14.46% and 2.19%, respectively. However, transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance (gs) decreased in Si-treated plants. These parameters decreased by 15.8%, 26.16%, 23.76% in 0.32 g Si treatment compared to 0 g Si and by 10.30%, 15.94% and 18.18% respectively in 0.16 g Si treatment (Table 1).

Under ambient CO_2 with BPH infestation, photosynthetic rate and WUE significantly increased with Si amendment by 3.63% and 46.4%, in 0.32 g Si treatment compared to 0 g Si and by 4.7% and 55.08%, respectively in 0.16 g Si treatment. However, transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance (gs) decreased in Si-treated plants by 21.62%, 32.83%, and 14.09% in 0.32 g Si treatment compared to 0 g Si and by 14.70% (increased), 30.94% (decreased) and 6.91% (decreased) respectively, in 0.16 g.

Under elevated CO_2 without BPH infestation, photosynthetic rate and WUE significantly increased with Si amendment by 26.15% and 0.80%, respectively in 0.32 g Si treatment compared to 0 g Si and, by 14.52% and 8.18%, respectively in 0.16 g Si treatment. However, transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance (gs) decreased in Si treated plants by 15.9%, 15.7%, 30.08% in 0.32 g Si treatment compare to 0 g Si, by 4.26%, 18.87% and 10.12% respectively, in 0.16 g Si treatment (Table 1).

Under ambient CO_2 without BPH infestation, photosynthetic rate and WUE significantly increased with Si amendment by 52.43% and 11.11%, respectively in 0.32 g Si treatment compared to 0 g Si and, by 6.04% (increased) and 14.43% (decreased), respectively in 0.16 g Si treatment. However, transpiration rate (E), intercellular CO_2 concentration (Ci), and stomatal conductance (gs) decreased in Si-treated plants, by 18.09%, 15.88%, 14.26% in 0.32 g Si treatment compare to 0 g Si and by 7.62% (decreased), 20.43% (increased) and 7.4% (decreased) respectively, in 0.16 g Si treatment (Table 1).

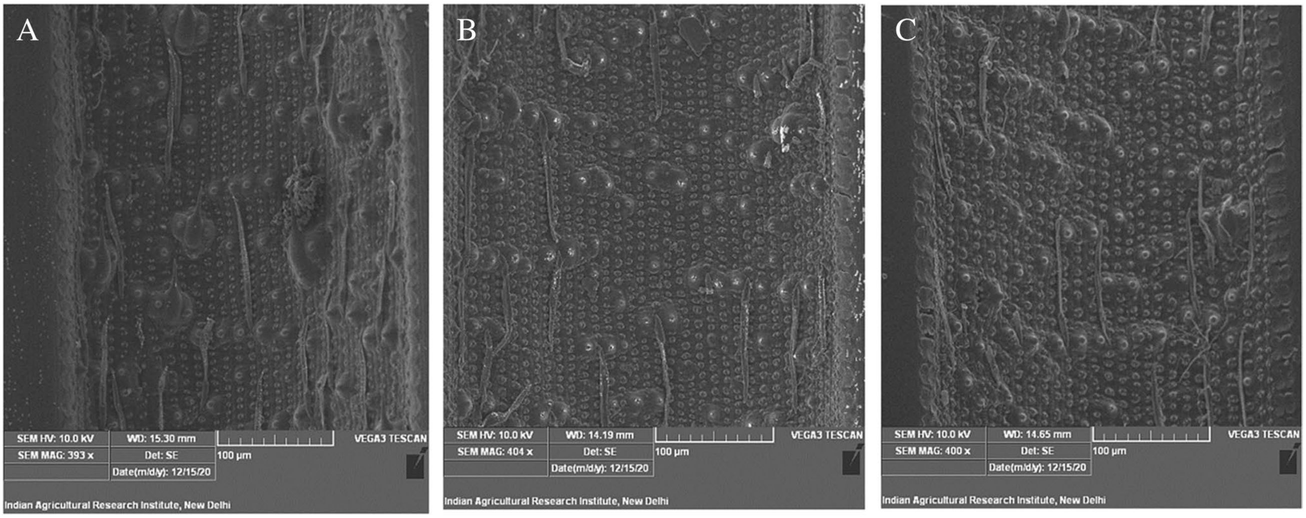


Fig. 5 Scanning Electron Microscope (SEM) pictures of silica bodies and silica cells on the leaf sheath of rice plant treated with silicon at the rate 0 g (**A**), 0.16 g (**B**), and 0.32 g (**C**) per kg soil

Table 1 Effect of elevated CO_2 and Silicon amendment on photosynthesis and related parameters in rice (IRGA analysis)

Treatment	Photosynthetic rate (P) (μmol CO_2 m^{-2} s^{-1})	Transpiration rate (E) (mmol H_2O m^{-2} s^{-1})	Water use efficiency (WUE) (mmol CO_2 mol^{-1} H_2O)	Intercellular CO_2 concentration (Ci) (mmol CO_2 m^{-2} s^{-1})	Stomatal conductance (gs) (mmol H_2O m – 2 s – 1)
Elevated CO_2 + 0 g Si + BPH	20.44 ± 0.68 cd	7.45 ± 0.27bcde	3.05 ± 0.08b	218.08 ± 0.65ab	258.55 ± 3.52d
Elevated CO_2 + 0.16 g Si + BPH	22.19 ± 0.67c	6.99 ± 0.34de	3.19 ± 0.10a	191.58 ± 0.34d	240.93 ± 7.86e
Elevated CO_2 + 0.32 g Si + BPH	25.40 ± 0.67ab	6.27 ± 0.34e	3.26 ± 0.03a	161.03 ± 1.12f	197.11 ± 3.14 g
Elevated CO_2 + 0 g Si - BPH	20.38 ± 0.48 cd	7.48 ± 0.27bcde	2.49 ± 0.03c	214.17 ± 0.13bc	318.28 ± 5.82b
Elevated CO_2 + 0.16 g Si - BPH	22.45 ± 0.61bc	6.57 ± 0.10de	2.32 ± 0.06d	222.54 ± 0.89a	247.28 ± 2.14e
Elevated CO_2 + 0.32 g Si - BPH	25.71 ± 0.75a	6.29 ± 0.38e	2.51 ± 0.05c	180.54 ± 0.71e	222.25 ± 5.53f
Ambient CO_2 + 0 g Si + BPH	12.66 ± 0.71e	9.25 ± 0.17a	1.25 ± 0.03 h	216.75 ± 0.12abc	346.90 ± 10.12a
Ambient CO_2 + 0.16 g Si + BPH	12.53 ± 0.85e	8.50 ± 0.33ab	1.18 ± 0.01 h	210.81 ± 1.71c	320.22 ± 8.71b
Ambient CO_2 + 0.32 g Si + BPH	13.12 ± 0.57e	7.25 ± 0.42cde	1.83 ± 0.10e	145.57 ± 2.13 g	298.08 ± 6.21c
Ambient CO_2 + 0 g Si - BPH	13.12 ± 0.23e	9.55 ± 0.48a	1.44 ± 0.11 g	192.18 ± 0.67d	353.28 ± 6.45a
Ambient CO_2 + 0.16 g Si - BPH	18.86 ± 0.64d	8.39 ± 0.60abc	1.87 ± 0.01e	133.43 ± 0.34 h	327.14 ± 7.10b
Ambient CO_2 + 0.32 g Si - BPH	20.00 ± 0.54 cd	7.75 ± 0.67bcd	1.60 ± 0.03f	160.69 ± 0.55f	302.90 ± 4.13c
F-value	45.41	20.52	159.9	633.99	577.52
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

4 Discussion

The rise in atmospheric CO_2 level and the temperature has an immediate impact on food grain production as well as an indirect effect on crop pests [8]. Generally, an increase in atmospheric CO_2 increases the C:N ratio of the rice plant foliage, and as a result, the pest increases the consumption under elevated CO_2 to compensate for lower nitrogen in plant foliage. As a result, the Si amendment can be used as a potential alternative management practice to reduce pest damage under elevated CO_2 levels. From the previous studies [28, 33] it is evident that the Si amendment impairs the sucking behaviour of BPH in rice, that was tested in the present study under elevated and ambient CO_2 condition. Our results also confirmed the results of Yang et al. [28] that Si amendment reduced the honeydew excretion, indicating reduced feeding by BPH. Honeydew excretion increased as the BPH infestation duration increased. Comparatively, slightly higher honeydew excretion was observed under elevated CO_2 compared to the ambient CO_2. However, honeydew excretion was found significantly lower at higher doses of Si compared to the lower dose or no Si treatment. The plant physiological modification behind the reduced honeydew excretion and feeding was studied at the molecular level in separate experiments. The callose deposition in the sieve tube of rice leaf sheath in response to BPH feeding was recorded through histological observations. The callose deposition responded positively to BPH infestation in plants both with and without Si amendment and more callosic sieve plates were noticed in the BPH-infested plants than uninfested plants. The lower honeydew excretion at different BPH infestation durations that corresponded to higher callose deposition in the sieve tubes of rice plants indicated about the probable relationship between callose deposition and reduction in BPH feeding. In resistant rice varieties closing or occlusion of phloem sap by callose deposition in sieve tubes in response to the BPH infestation has already been demonstrated [23], indicating that the Si amendment might have played a role in reducing BPH feeding. Unlike silicification, callose deposition is an induced defense by the Si-treated plants. The deposition of callose, a polysaccharide, is regulated by the expression of genes encoding callose synthase (*OsGSL1*) and callose hydrolase (*Gns5*) [23]. Our study found that BPH infestation induced high expression of OsGSL1 and low expression of Gns5 in plants treated with Si, compared to those without Si amendment. This led to greater callose deposition in Si-treated plants, resulting in reduced feeding and lower honeydew excretion by BPH, compared to Si-untreated plants [28, 33]. However, after the initial infestation period (24 and 48 hpi), both genes' expression decreased in both Si-treated and untreated plants, and the number of callosic sieve plates tended to decline from 72 hpi onwards. This dynamic expression of *OsGSL1* and *Gns5* genes, and the accumulation of callose, is a response to herbivore stress. Previous research suggests that prolonged callose deposition can adversely affect plant physiology and development, making its short-lived deposition under herbivore stress conditions beneficial [28].

Soil application of Si and uptake of available Si by plant significantly alters the physiology of the plant and also the nutrient composition of soil and plant tissue [34]. In this study, we found that Si amendment significantly increased the photosynthetic rate and WUE and decreased transpiration rate (E), intercellular CO_2 concentration (Ci), and stomatal conductance (gs) in rice plants, both under elevated and ambient CO_2 conditions, with and without BPH infestation. This increase in photosynthesis might be attributed to the enhanced activity of antioxidant enzymes, RuBP carboxylase, and an increase in chlorophyll content, as reported previously [35]. Our results are in line with other studies that have found that Si application can increase the net photosynthesis rate in various crops, such as wheat [36, 37], sorghum [38], and soybean [39]. The lowest transpiration rate observed in Si-treated plants could be due to decreased stomatal activity. It has been suggested that Si plays an important role in decreasing the transpiration rate, which helps to protect the moisture content of the plants [40]. Si application has also been found to significantly decrease transpiration and increase WUE in wheat [41] and maize [42] plants. However, in contrast to previous reports in other crops [37], our study found that Si application significantly decreased stomatal conductance. The Si amendment also enhanced the accumulation of Si in plant tissues, with higher doses resulting in higher accumulation levels under ambient and elevated CO_2 conditions.

5 Conclusion

Si amendment increased the accumulation of silica cells on leaf sheath, expression of resistance-inducing genes, increased the callose deposition, and reduced honeydew excretion or the pest feeding. It also altered the photosynthetic parameters in a beneficial way, modified the soil's nutrient composition, and increased the available form of major and minor nutrients including Si. All these results revealed the implication of the Si application as a potential alternative strategy to combat rice BPH under both elevated and ambient CO_2 and temperature.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12633-023-02480-w.

Acknowledgements We thank the Division of Entomology, Division of Genetics and Division of plant physiology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India for providing the necessary facilities for the conduct of experiments.

Author Contributions Author contributions: All authors contributed to the study conception and design. Prabhulinga Tenguri, Subhash Chander, Ranjith K Ellur, Arya P S, Yogesh Yele, Madhu T N, S.Subramanian and Sachin S. Suroshe : Material preparation, data collection and analysis. Prabhulinga Tenguri and Subhash Chander: the first draft of the manuscript. All authors read and approved the final manuscript.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Sogawa K, Liu GJ, Shen JH (2003) A review on the hyper-susceptibility of Chinese hybrid rice to insect pests. Chin J Rice Sci 17:23–30
2. Pathak MD, Saxena RC (1980) Breeding approaches in rice. In: Smith H (ed) Breeding plants resistant to insects. Pergamon, Oxford, pp 61–81
3. Cabauatan PQ, Cabunagan RC, Choi IR (2009) Rice viruses transmitted by the brown planthopper *Nilaparvata lugens* (Stal). Plant hoppers: new threats to the sustainability of intensive rice production systems in Asia (ed. by KL Heong & B Hardy). International Rice Research Institute, Los Baños, pp 357–368
4. Brar DS, Virk PS, Jena KK, Khush GS (2009) Breeding for resistance to planthoppers in rice. In: Heong KL, Hardy B (eds) *Plant hoppers* New Threats to the sustainability of Intensive Rice Production Systems in Asia. International Rice Research Institute, Los Baños, pp 401–427
5. Prasannakumar NR, Chander S, Sahoo RN, Gupta VK (2013) Assessment of brown planthopper, Nilaparvata lugens (Stal.), damage in rice using hyperspectral remote sensing. Int J Pest Manag 59(3):180–188. https://doi.org/10.1080/09670874.2013.808780
6. Prahalada GD, Shivakumar N, Lohithaswa HC, Sidde Gowda DK, Ramkumar G, Kim SR, Ramachandra C, Hittalmani S, Mohapatra T, Jena KK (2017) Identification and fine mapping of a new gene, BPH31 conferring resistance to brown plant hopper biotype 4 of India to improve rice. Oryza sativa L Rice 10(1):1–15. https://doi.org/10.1186/s12284-017-0178-x

7. Bae SH, Pathak MD (1970) Life history of *Nilaparvata lugens* (Homoptera: Delphacidae) and susceptibility of rice varieties to its attacks. Ann Entomol Soc Am 63(1):149–155. https://doi.org/10.1093/aesa/63.1.149
8. Coakley SM, Scherm H, Chakraborty S (1999) Climate change and disease management. Annu Rev Phytopathol 37:399–426. https://doi.org/10.1146/annurev.phyto.37.1.399
9. Xiao-Na CHEN, Wei H, Neng-Wen XIAO, Jun-Sheng L, Lan-Zhi H, Fa-Jun C (2011) Effects of elevated CO_2 and transgenic bt rice on yeast like endosymbionts and its host brown planthopper. J Appl Entomol 135(5):333–342. https://doi.org/10.1111/j.1439-0418.2010.01558.x
10. Chen FJ, Wu G, Ge F (2004) Impacts of elevated CO_2 on the population abundance and reproductive activity of aphid *sitobion avenae* feeding on spring wheat. J Appl Entomol 128(9–10):723–730. https://doi.org/10.1111/j.1439-0418.2004.00921.x
11. Sudderth EA, Stinson KA, Bazzaz FA (2005) Host-specific aphid population responses to elevated CO_2 and increased N availability. Glob Chang Biol 11:1997–2008. https://doi.org/10.1111/j.1365-2486.2005.01006.x
12. Xie H, Zhao L, Wang W, Wang Z, Ni X, Cai W, He K (2014) Changes in life history parameters of *Rhopalosiphum maidis* (Homoptera: Aphididae) under four different elevated temperature and CO_2 combinations. J Econ Entomol 107(4):1411–1418. https://doi.org/10.1603/EC13302
13. Pandi GP, Chander S, Singh MP, Pathak H (2018) Impact of elevated CO_2 and temperature on brown planthopper population in rice ecosystem. Proc Natl Acad Sci India Sect B Biol Sci 88(1):57–64. https://doi.org/10.1007/s40011-016-0727-x
14. Badiani MD, Annibale A, Paolacci AR, Miglietta F, Raschi A (1993) The antioxidant status of soybean (*Glycine max*) leaves grown under natural CO_2 enrichment in the field. Aust J Plant Physiol 20(3):275–284
15. Tenguri P, Chander S, Ellur RK, Arya PS, Yele Y (2023) Deciphering host plant resistance mechanisms of rice genotypes resistant against Brown Planthopper. Euphytica 219:8. https://doi.org/10.1007/s10681-022-03136-3
16. Yele Y, Chander S, Suroshe SS, Nebapure SM, Arya PS, Tenguri P (2021) Effect of ecological engineering on incidence of key rice pests. Indian J Entomol 84(3):503–508. https://doi.org/10.55446/IJE.2021.94
17. Han Y, Li P, Gong S, Yang L, Wen L, Hou M (2016) Defense responses in rice induced by silicon amendment against infestation by the leaf folder *Cnaphalocrocis medinalis* PLoS ONE 11(4):e0153918. https://doi.org/10.1371/journal.pone.0153918
18. Massey FP, Ennos AR, Hartley SE (2006) Silicon in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. J Anim Ecol 75:595–603. https://doi.org/10.1111/j.1365-2656.2006.01082.x
19. Han YQ, Lei WB, Wen LZ, Hou ML (2015) Silicon-mediated resistance in a susceptible rice variety to the rice leaf folder, *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Pyralidae). PLoS ONE 10:e0120557. https://doi.org/10.1371/journal.pone.0120557
20. Hartley SE, Fitt RN, McLarnon EL, Wade RN (2015) Defending the leaf surface: intra- and inter-specific differences in silicon deposition in grasses in response to damage and silicon supply. Front Plant Sci 6:35–35. https://doi.org/10.3389/fpls.2015.00035
21. Kvedaras OL, An M, Choi YS, Gurr GM (2010) Silicon enhances natural enemy attraction and biological control through induced plant defenses. Bull Entomol Res 100:367–371. https://doi.org/10.1017/S0007485309990265
22. Tenguri P, Chander S, Nebapure S, Arya PS, Madhu TN, Yogesh Yele (2022) Effect of silicon amendment on herbivore induced plant volatiles of rice plant infested by Brown Planthopper *Nilaparvata lugens* (STÅL). Indian J Entomol 1–4. https://doi.org/10.55446/IJE.2022.595
23. Hao P, Liu C, Wang Y, Chen R, Tang M, Du B, Zhu L, He G (2008) Herbivore-induced callose deposition on the sieve plates of rice: an important mechanism for host resistance. Plant Physiol 146(4):1810–1820. https://doi.org/10.1104/pp.107.111484
24. Heinrichs EA, Medrano FG, Rapusas HR (1985) Genetic evaluation for Insect Resistance in Rice. International Rice Research Institute, Los Baños, pp 1–355
25. Pathak PK, Saxena RC, Henrichs EA (1982) Parafilm sachet for measuring honeydew excretion by Nilaparvata lugens on rice. J Econ Entomol 75:194–195. https://doi.org/10.1093/jee/75.2.194
26. Du B, Zhang WL, Liu BF, Hu J, Wei Z, Shi ZY, He RF, Zhu LL, Chen RZ, Han B, He GC (2009) Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. PNAS 106:22163–22168. https://doi.org/10.1073/pnas.0912139106
27. Kim BR, Nam HY, Kim SU, Kim SI, Chang YJ (2003) Normalization of reverse transcription quantitative-PCR with housekeeping genes in rice. Biotechnol Lett 25(21):869–1872. https://doi.org/10.1023/A:1026298032009
28. Yang L, Li P, Li F, Ali S, Sun X, Hou M (2018) Silicon amendment to rice plants contributes to reduced feeding in a phloem-sucking insect through modulation of callose deposition. Ecol Evol 8(1):631–637. https://doi.org/10.1002/ece3.3653
29. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 – ΔΔCT method. Methods 25(4):402–408
30. McNairn RB, Currier HB (1967) Sieve plate callose. A factor in blockage of axial phloem transport. Naturwissenschaften 54:591. https://doi.org/10.1007/BF00636832
31. R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
32. Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. Wiley-Interscience, New York
33. Yang L, Han Y, Li P, Wen L, Hou M (2017) Silicon amendment to rice plants impairs sucking behaviors and population growth in the phloem feeder *Nilaparvata lugens* (Hemiptera: Delphacidae). Sci Rep 7(1):1–7. https://doi.org/10.1038/s41598-017-01060-4
34. Pavlovic J, Kostic L, Bosnic P, Kirkby EA, Nikolic M (2021) Interactions of silicon with essential and beneficial elements in plants. Front Plant Sci 12:697592. https://doi.org/10.3389/fpls.2021.697592
35. Ma JF, Tamai K, Ichii M, Wu K (2002) A rice mutant defective in active Si uptake. Plant Physiol 130:2111–2117
36. Gong H, Zhu X, Chen K, Wang S, Zhang C (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci 169:313–321. https://doi.org/10.1016/j.plantsci.2005.02.023
37. Jeer M, Yele Y, Sharma KC, Prakash NB (2020) Exogenous application of different Silicon sources and Potassium reduces Pink Stem Borer damage and improves photosynthesis, yield and related parameters in wheat. Silicon 13:901–910. https://doi.org/10.1007/s12633-020-00481-7
38. Ahmed M, Hassen FU, Qadeer U, Aslam MA (2011) Silicon application and drought tolerance mechanism of sorghum. Afr J Agric Res 6:594–607. https://doi.org/10.5897/AJAR10.626
39. Shen X, Zhou Y, Duan L, Li Z, Eneji AE, Li J (2010) Si effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J Plant Physiol 167:1248–1252. https://doi.org/10.1016/j.jplph.2010.04.011
40. Ma JF, Goto S, Tamai K, Ichii M (2001) Role of root hairs and lateral roots in silicon uptake by rice. Plant Physiol 127:1773–1780
41. Bybordi A (2015) Influence of exogenous application of silicon and potassium on physiological responses, yield, and yield components of salt- stressed wheat. Commun Soil Sci Plant Anal 46:109–122. https://doi.org/10.1080/00103624.2014.956936

42. Gao X, Zou C, Wang L, Zhang F (2006) Silicon decreases transpiration rate and conductance from stomata of maize plants. J Plant Nutr 29:1637–1647. https://doi.org/10.1080/01904160600851494