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Silicon induced resistance in finger millet (*Eleusine coracana* L.) against pink stem borer, *Sesamia inferens* (Walker) through modulation of defense responses

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Abstract
Silicon confers stress either through physical barrier or by triggering the chemical resistance mechanism, but our understanding to silicon mediated plant resistance to pink stem borer through physiological and cytological mechanisms is underlying. Present investigation highlight silicon induced resistant against the pink stem borer, *Sesamia inferens* (Walker) in ragi plants. Si amendment to ragi plants much reduced the feeding ability of pink stem borer through modulation of leaf sheaths silicification. It also induces the chemical defense system by influencing the defense-related enzymes, syntheses of secondary metabolites, and concentrations of malondialdehyde, total phenol and soluble protein in a leaf sheath of infested susceptible (Suvra) and resistant (HR-379) ragi plants. Inclusion of silicon encouraged the increase of H_2O_2 concentration and suppressed the malondialdehyde concentration in both infested susceptible and resistant varieties. Superoxide dismutase, catalase and peroxidase activities were higher in both the varieties amended with silicon than in non-amended stem borer infested plants. Stem borer infestation activated synthases for secondary metabolites, phenylalanine ammonia-lyase, β-1,3-glucanase and total phenol in silicon amended plants, but performance of polyphenol oxidase and soluble protein content was lower in Si-amended plants than in non-amended plants. The present study also indicates that Si amendment interacts with stem borer infestation to confer enhanced finger millet resistance to pink stem borer by priming the reduction in soluble protein content and cell silicification of leaf sheaths, and induction of plant defense responses.

Keywords: Finger millet, stem borer, silicon, biotic stress

Introduction
Crop damage either partly or fully made through both insect pests. There are over a dozen pests, which, when infest the crop, do damage the crop to a varying degree depending upon the crop growth (Puttaswamy and Channallasavanna, 1977) [33]. Herbivorous insects are responsible to destroy one-fifth of the world's total crop production annually. However, few of them are severe to cause economic losses. Stem borers is one of a destructive group of insects attacking millets. There are three stem boring caterpillars- pink borer, white borer and sorghum stem borer- in central and southern India. Pink stem borer is known for its polyphagous behavior and feeds on sorghum, maize, rice, wheat, sugarcane, ragi, barley and guinea grasses. *Sesamia inferens* is one of the major insects pest of millets and equally responsible for damaging millets in India and Africa **(**Krishnamurti and Usman, 1952; Tams and Bowden, 1953) [18, 46]. It is widely distributed in all finger millet growing countries. In India, it is more regularly recorded from Orissa, Karnataka, Tamil Nadu and Andhra Pradesh. The damage starts from the seedling stage and continues till maturity. Stem boring resulted profuse tillering with unproductive spikes. Late attacks result in peduncle damage and plant breakage which also contributes the losses of grains and fodder. In this context, to check the yield loss due to stem borer (SB) infestation, application of chemical control, which is expensive, and leads to damage to environment particularly the target species (Pimentel and Burgess,2012) [31]. In addition, overuse of pesticides destroys natural enemies and leads to the insect developing resistance, which results in pest resurgence (Li *et al*. 2011) [23]. Therefore, it is very essential to develop effective and ecofriendly alternative method to improve the pest control. Silicon (Si) a silver bullet indicates one of such potential alternatives for insect pest management (Hou and Han, 2010; Savant *et al*. 1997) [14, 38].

Silicon amendment increases rigidity and reduced digestibility of plant tissue due to more amorphous silica deposition, and then enhance constitutive defense in Si amended (Hou and Han, 2010; Reynolds *et al*. 2009; 2016) [14, 36, 37] and augmenting the release of herbivore induced plant volatiles that attract natural enemies of harmful pests **(**Kvedaras *et al*. 2010; Yang *et al.* 2017) [19, 50]. The goal of this study was to explore Si-mediated defense responses in ragi plants to SB infestation to explain the physiological mechanisms for the enhanced plant resistance associated with Si amendment.

Materials and Methods

Selection of genotypes

The experiment based on selecting both susceptible (Suvra) and resistance (HR-379) varieties of ragi. After germination, the germinated seeds were sown in plastic pots (27×19 ×4 cm) with 50% vermiculite and 50% Coco peat where the plants were supplemented with Hoagland nutrient. After sixty days, single seedling was transplanted to each plastic pot (50×40 ×15 cm) for further experiment. The nutrient medium adjusted to pH 6.0. Si amendment (+Si) by adding Silicic acid to the nutrient solution at 1mM Si/l, a control without addition of silicic acid (−Si). The nutrient solution was replenished every five days. The plants were cultured in a greenhouse to prevent rain and natural occurring pests. Pesticides were not used throughout the experiment.

Stem borer infestation

Collection of pink stem borer larvae from ragi fields at AICRP-Small millet centre (Berhampur, Odisha, India). The larvae raised on susceptible variety "Suvra" until pupa formation. Pupas incubated in plastic jar covered with muslin cloth for adult emergence under the control condition in growth chamber. After emergence the adults to caged susceptible ragi plants in the growth chamber for oviposition. Maintained a stock culture of the newly hatched first instars on ragi plants without Si addition in a growth chamber until the larvae there reached the third instars at 28±1 °C, 70 ± 5% relative humidity and 16h photoperiod.

Sample collection

Four treatments were given to the plant. Treatment-1 with Si addition and stem borer infestation (+Si+SB); Treatment-2 without Si addition and with SB infestation (-Si+SB); Treatment-3 with Si addition and without SB infestation (+Si-SB); Treatment-4 without Si addition and SB infestation (-Si-SB). Samples of leaf sheaths collected at 0, 24, 48, 72 and 192 h post-infestation (hpi) times and stored at −80 °C. The leaf sheath samples used to measure the activities of antioxidant enzymes (CAT, POD and SOD), syntheses of secondary metabolites (PPO and PAL), β-1,3-glucanase, concentrations of MDA, H_2O_2, soluble protein and total phenol content.

Scanning electron microscopy and energy dispersive X-ray analysis

Relative Si content and silica deposition on the surface of leaf sheath of susceptible variety after 24hpi under scanning electron microscopy coupled with EDX microanalysis mapping. The small section of leaf sheath stored in the refrigerator to avoid sample drying for SEM-EDX analysis. The specimens examined under a Scanning Electron Microscope (Hitachi S3400N, Japan) without metal sputtering. Image taken in all specimens under high vacuum at 20 kV, with 13.6 mm working distance and a 200 µm objective lens aperture. This technology relies on atomic excitation by electron beams, which provides a semi-quantitative determination of nutrient content by proportionality of scanned area (McMullan, 2006) [27].

Assessment of enzyme activity

The frozen leaf sheath samples (5gm) of both the treated and untreated varieties were homogenized in pre-chilled mortar and pestle with 150 mM phosphate buffer saline (PBS, pH 7.8), containing 1 mM EDTA, at a 1:6 ratio (fresh weight of sample/buffer volume). The crude homogenates were centrifuged at 14,000 rpm for 20 min at 4 °C (Centrifuge 5430 R, Eppendorf, Hamburg, Germany). The supernatant further used to determine the activities of Catalase (CAT), Superoxide dismutase (SOD), Peroxidase (POD), Polyphenol oxidase (PPO) and Polyphenol oxidase (PAL).

Catalase activity

Estimation of Catalase (CAT, EC 1.11.1.6) activity by the UV Spectrophotometric method (Aebi, 1983) [1]. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 100 µL enzyme sample. The increase in absorbance at 240 nm recorded for 3 min at 25 °C. Specific activity of enzyme expressed as mmoles H_2O_2 decomposed mg^{-1} protein min^{-1} by using the H_2O_2 extinction coefficient of 39.4 mM^{-1} cm^{-1}.

Superoxide dismutase activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity assayed according to the method of Giannopolitis and Ries (1977) [10]. The reaction mixture contained 0.1 ml the enzyme preparation, 50 mM sodium phosphate buffer (pH 7.8), 9.9 mM L-methionine, 2.0 mM EDTA, 55 µM Nitro-Blue-Tetrazolium (NBT) and 1.0 µM riboflavin. The activity was measured at 560 nm. Absorbance of enzyme extracts volume corresponding to 50% inhibition of the photochemical reaction obtained and considered as one enzyme unit. The enzyme activity was estimated as unit per mg^{-1} protein.

Peroxidase (POD) activity

Peroxidase (POD, EC 1.11.1.7) activity estimated according to the method of Putter (1974) [32]. In the test cuvette, the reaction mixture consisting of 3.0 ml of phosphate buffer (0.1 M, pH 7.0), 50 µl guaiacol solution (20 mM), 100 µl enzyme sample and 30 µl H_2O_2 solution (12.3 mM). The rate of formation of GDHP was analyzed spectrophotometrically at 436 nm. One unit of enzyme activity defined as the amount of enzyme catalyzing for 1.0 µM of GDHP/min/g fresh weight.

Polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO, EC 1.14.18.1) activity determined according to the method of Dawson and Magee (1955) [7]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 6.5), 5 mM L-3,4-Dihydroxyphenylalanine (L-DOPA), 2.1 mM L-Ascorbic acid, 0.065 mM EDTA and 100 µl enzyme extracts. One unit of PPO activity was deemed as the amount of enzyme caused the change of 0.001 at 265 nm per min at 25 °C.

Phenylalanine ammonia-lyase (PAL) activity

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity assayed by the method of Schopfer (1971) [39]. Assay of PAL activity was made by incubating 0.5 ml 0.01M L-

phenylalanine, 1 ml 0.05M Tris-HCl buffer (pH 8.8) at 37 °C for 1h. About 0.5 ml 1N HCl was added to stop the reaction. Extracted the mixture twice with 3.5 ml of ether and subsequently dried under stream of air. The residue was dissolved in 3 ml of 0.05N NaOH. Similarly, standard curve was made by using the cinnamic acid. The enzyme activity expressed in micromole of cinnamic acid produced/min/mg protein.

β-1,3-glucanase activity

β-1,3-glucanase (GNS, EC 3.2.1.58) activity assayed by measuring the amount of reducing sugar released from the laminarin (Sigma L-9634) substrate according to the dinitrosalicylic acid (DNS) method. The reaction mixture was made by adding of 100 µl of crude protein extract with 100 µl of 2% (w/v) laminarin and incubated at 37 °C for 1h. About 1 ml of 1% (v/v) staining DNS reagent was added and boiling for 5 min to stop the reaction. After cooling at room temperature, the content was diluted to 1:10 in deionizer water. The absorbance was taken at 500 nm. Enzyme activity was expressed in nmol as amount of released reducing sugar (D-glucose) per hour per miligram of soluble protein.

MDA, H_2O_2, Phenol and soluble protein content

Malondialdehyde (MDA) determination was made by using the method of Carmak and Horst (1991) [4]. About 0.5g of fresh leaf tissue was homogenized in 2ml 0.1% (w/v) trichloroacetic acid (TCA) at 4 °C and centrifuged at 20,000 x g for 15 min. After centrifugation, 0.5 ml supernatant was added in 3 ml 0.5% (v/v) thiobarbituric acid (TBA) and incubated the mixture at 95 °C in a shaking water bath for 50 min. Subsequently, the mixture was kept in the ice to stop the reaction. Further, the measurement was made at 532 nm and subtracted the value for nonspecific absorption at 600 nm. The concentration of MDA as expressed as nmol g^{-1} fresh weight.

Estimation of hydrogen peroxide (H_2O_2**)** content by the method of Noreen and Ashraf (2009) [29]. Fresh leaf tissue (0.1 g) homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) in pestle and mortar and centrifuged at 12,000×g for 15 min. Further, add 0.5 ml of supernatant, 0.5 ml of phosphate buffer (pH 7.0) and 1 ml of 1M potassium iodide (KI) and taken absorbance at 390nm. H_2O_2 concentration expressed as µmol g^{-1} FW (extinction coefficient of H_2O_2 0.28 µM cm^{-1}).

For estimation of phenolic, 500 mg of fresh leaves homogenized in 3 ml of 80% (v/v) methanol and agitated for 15 min at 70 °C and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and used for estimation of total phenols. Further, 2 ml of 2% sodium carbonate (Na_2CO) taken in a test tube and add 1 ml of extract, incubated for 5 min at room temperature. After incubation, 0.1 ml of 1N Folin-Ciocalteu reagent was added and re-incubated for 10 min to obtain blue colour. The absorbance was measured absorbance at 760 nm. Phenolic concentration expressed as mg catechol equivalents g^{-1} FW (mg GAE g^{-1} fresh weight).

Protein content in the enzyme extracts estimated using the Lowry *et al.* (1993) [22] method, bovine serum albumin was used as a standard. Specific activity of all the enzymes expressed as units per milligram protein.

Data analysis

All data in figures shown as means ± SE. The data of enzyme activities and concentrations of MDA, H_2O_2, phenol and soluble protein subjected to three-way analysis of variance (ANOVA) for the effects of Si amendment, stem borer infestation, stem borer infestation time and the interactions between the three treatments. Three way statistical analyses performed using Graph Pad Prism 7 (California, USA) followed by Tukey's multiple range test ($P = 0.05$) or by independent-samples T-test for separation of mean significant differences between treatments performed using XLSTAT 2018 (Addinsoft, France).

Results

Silicon amendment and stem borer infestation.

Based on scanning image analysis of leaf sheath, it observed that the silica deposition in leaf sheath of susceptible variety "Suvra", after 24 hours of post infestation look likes dumbbell shape-like structures and sparsely distributed (Fig.1). The results showed that silica cells varied among treatments. The SB infestation and Si addition led to intensive cell silicification in ragi leaf sheath. Percentage of silicon deposition was 0.3% in silicon amended infested plants followed by non-infested plants 0.19 weight percent deposition. No difference observed in –Si + SB and –Si –SB plants, and showed 0.10 and 0.08 weight percent of silicon deposition.

Enzymatic Activity to Si amendment and SB infestation

SOD activity: For susceptible "Suvra" variety, ANOVA showed that Si addition, and SB infestation influenced SOD activity in leaf sheath but SB infestation time showed insignificant (Table 1). Meanwhile, for resistant variety "HR-379", Si addition and SB infestation time was much influenced but not the SB infestation (Table 2). The interaction between all the factors in both the varieties had significant effect except SB infestation, and SB infestation time in resistant and susceptible varieties (Table 1 and 2). Without SB infestation, SOD activity was significant between +Si and -Si a time point 0 – 48h post infestation (Fig.2A; $t \geq 6.814$, $P \leq 0.036$) in susceptible, and 0, 48, 192 hpi (Fig.2B; $t \geq -8.885$, $P \leq 0.037$) in resistant varieties. In SB infested plants, SOD activities showed a sharp increased pattern for both the varieties, but at the different time points. SOD activity in susceptible variety was highest at 24hpi. Later, the activity decreases in susceptible variety from 24 to 192 hpi in +Si treated plants but in – Si treated plants, the activity increases in resistant variety from 48 to 192hpi over an un-infested plants ($F \geq 11.731$, df = 3, 7, $P \leq 0.000$). Silicon amendment added to the increase in SOD activity in susceptible and resistant infested plants, which shows by higher increases in +Si plants than in -Si plants by 13.1% and 63.4% at 24 and 48hpi. However, decreases at 24, 48 and 72 hpi in susceptible and resistant plants.

CAT activity. Si addition, SB infestation and SB infestation time, and their interactions between these three factors much influenced CAT activity in both susceptible and resistant varieties except an infestation time in resistant variety (Table 1and 2). In un-infested plants, Si addition did not affect CAT activity in susceptible variety at all-time points except at 24hpi (Fig 2C; t=-5.752, P=0.029). In case of 24hpi, the CAT activity less as compared to –Si plants. Meanwhile, in resistant variety, the CAT activity was more at all-time points and decreased as compared to –Si plants (Fig. 2D; $t \geq -13.337$, $P \leq 0.012$). In stem borer infested susceptible variety, CAT activity increases in both +Si and –Si plants compared with

un-infested plants except at 0 hpi (hour post infestation) ($F \geq 194.003$, df = 3, 7, $P \leq 0.0001$). Similarly, in resistant variety, CAT activity was also increased in both +Si and –Si plants over un-infested plants ($F \geq 8.615$, df = 3, 7, $P \leq 0.032$). In both susceptible and resistant varieties, maximum CAT activity at 48 h post infestation in +Si+SB plants but less in −Si +SB susceptible variety which was below the un-infested plants. In susceptible infested plants, CAT activity was higher in +Si plants than in –Si plants at all-time points except at 0 hpi. The highest activity noted at 48h (429.8%) followed by 72h (183.4%), 24h (166.3%) and 192h (22.2%) post infestation ($t \geq 10.588$, $P \leq 0.009$). Similarly, in resistant infested plants, CAT activity was much higher in +Si plants as compared to –Si plants at 48h (237%) followed by 0h (148%) and 72h (85.8%) post infestation ($t \geq 8.485$, $P \leq 0.014$).

POD activity: Si addition, SB infestation, and SB infestation, and all interactions between these three factors appreciably influenced POD activity in both susceptible and resistant varieties except an interaction between silicon amendment and infestation time in resistant variety (Table 1 and 2). In un-infested susceptible plants, Si addition alone did not change POD activity at all-time points, but in resistant plants the activity was significantly decreased as compared to –Si plants at all-time points (Fig. 1E; $t \geq -4.912$, $P \leq 0.039$). In infested plants of both the varieties, POD activity showed a temporal pattern with the highest peaked at 48hpi and 72hpi as compared to un-infested plants. Silicon amendment hiked POD activity in both susceptible and resistant infested varieties over non amendment silicon plants by 54.5%, 18.0% and 20.2% at 48, 72 and 192 h (Fig 2E; $t \geq 4.986$, $P \leq 0.038$) and by 21.3%, 26.6% and 37.4% at 0, 24and 48h post infestation, respectively (Fig. 1F; $t \geq 5.619$, $P \leq 0.030$).

Metabolites response to Si amendment and SB infestation

PPO activity

Si addition, SB infestation, SB infestation time and their interactions much affected PPO activity in both susceptible and resistant varieties except the interaction between infestation and infestation time (Table 1 and 2). In un-infested plants, PPO activity was decreased in silicon amended plants as compared to non-amended plants in both susceptible (Fig. 3A; $t \geq -32.714$, $P \leq 0.020$), and resistant (Fig. 3B; $t \geq -39.668$, $P \leq 0.001$) varieties. The highest PPO's activity shown in non-silicon amended plants at 24 and 48h sampling points in susceptible and resistant varieties. The change in rhythmic pattern observed in resistant variety with the highest peaks at the 48h and lowest at 24 and 72h sampling points. In case of susceptible plants with stem borer (SB), PPO activity showed more decreases as compared without Si treatment at all-time points except at 48h post infestation ($t \geq -31.726$, $P \leq 0.005$). Similarly, PPO activity in +SB resistant plants also observed with significant decrease in +Si treatment over -Si treatment at all-time points ($t \geq -65.054$, $P \leq 0.007$). Particularly, PPO activity in +Si+SB resistant plant was much lower than that in –Si –SB and –Si+SB plants at all-time points ($F \geq 708.373$, df = 2, 5, $P \leq 0.0001$). In both the +Si+SB varieties, PPO activity much reduced at all-time points except at 48h post infestation in susceptible variety by 21.3%, 16.6%, 18.4% and 47.1% and 36.5%, 36.6%, 45.6%, 46.9% and 16.0% in resistant variety as compared to –Si +SB treated plants.

PAL activity

PAL activity in both susceptible and resistant varieties was much higher by Si addition, SB infestation and SB infestation time, and all interactions between these three factors (Table 1 and 2). PAL activity decreased between –Si plants and +Si as compared to –Si in both un-infested susceptible (Fig. 3C; $t \geq -15.026$, $P \leq 0.026$) and resistant (Fig. 3D; $t \geq -11.741$, $P \leq 0.025$) plants. In both susceptible and resistant infested plants, the highest peaked of PAL activity at 24hpi and gradually decreased up to 192 hpi. In +Si +SB plants, PAL activity was much higher at all-time points ($t \geq 8.987$, $P \leq 0.012$) but decreased at 192hpi in susceptible plants compared with that in –Si +SB plants (t =-8.827, P =0.013). In resistant plants, PAL activity was also increased at all-time points over –Si+SB plants ($t \geq 7.304$, $P \leq 0.018$).In +Si+SB susceptible and resistant plants, PAL activity increased significantly by 17.6%, 24.1%, 7.9% and 66.0% at 0, 24, 48 and 72h post infestation and by 10.2%,27.2%,11.5%,42.5% and 30.9% at 0, 24, 48, 72 and 192h post infestation respectively, over that in +Si –SB plants.

Responses of plant MDA, and H_2O_2 contents to Si amendment and SB infestation

MDA concentration of both the varieties was much influenced by all the three treatments, and all their interactions (Table 1 and 2). Without SB infestation, the +Si and –Si plants of both the varieties was differed at all-time points of samplings. The silicon amendment decreases the MDA concentration over without Si amendment plants in susceptible (Fig.4A; $t \geq -41.453$, $P \leq 0.004$) as well as in resistant (Fig.4B; $t \geq -25.790$, $P \leq 0.007$) varieties at all-time points except 0h post infestation in resistant variety. Silicon amendment and treated with SB, MDA concentration showed increase at all-time points (except 0 hpi) of susceptible variety ($F \geq 4248.476$, df = 2, 5, $P \leq 0.0001$) and in resistant variety as compared to–SB plants. MDA concentration was much lower in without silicon and with stem borer (–Si +SB) plants at all samplings points by 10.5%, 33.1%, 38.1% and 20.0% ($t \geq -199.860$, $P \leq 0.002$) but increased at192h post infestation by 24.7% (t = 66.818, P $\leq$ 0.000) in susceptible plants. While in +Si+SB resistant plants, MDA concentration was lower at 24, 48 and 72 h post infestation by 63.7%, 35.1% and 23.0% ($t \geq -125.470$, $P \leq 0.0001$) and higher at 0 and 192h post infestation by 5.5% and 9.7% ($t \geq 11.648$, $P \leq 0.007$) than that in –Si +SB plants.

H_2O_2 concentration of both the varieties was also significant in all the three treatments and between their interactions except infestation time in susceptible variety (Table 1 and 2). Concentration of H_2O_2 in stem borer un-treated plants also showed the significant difference between +Si and –Si treated susceptible (Fig.4C; $t \geq -8.876$, $P \leq 0.039$) and resistant (Fig.4D; $t \geq -7.135$, $P \leq 0.050$) plants at all samples points. In between the treatments of +Si+SB and –Si+SB plants of both the varieties, H_2O_2 concentration increased in susceptible (Fig.3C; $t \geq 5.244$, $P \leq 0.034$) and resistant (Fig.4D; $t \geq 4.812$, $P \leq 0.041$) varieties at all-time points except at 192h post infestation. The highest peaked of H_2O_2 concentration in +Si+SB treatment at the different time points such as 48hpi in susceptible and 72 hpi resistant varieties over –Si+SB treatment. Meanwhile, in –Si+SB plants, the highest H_2O_2 at 192 h post infestation in both the varieties as compared to +Si+SB plants. In SB treated plants, the H_2O_2 concentration increased in silicon added plants by 110.8%, 90.7%, 24.3% and 78.2% in susceptible plants, and by 175.6%, 55.2%, 34.2 and 11.6% in resistant plants at 0, 24, 48 and 72h post infestation over that in –Si +SB plants.

β-1,3-glucanase,total phenol and soluble protein content in response to Si amendment and SB infestation

β-1,3-glucanase activity was much influenced in both the varieties by all three treatments viz. Si addition, SB infestation, SB infestation and their interactions (Table 1 and 2). The significant difference with decreased β-1,3-glucanase activity observed in +Si susceptible (Fig.5A; $t \geq -45.015$, $P \leq 0.002$) and resistant (Fig.5B; $t \geq -22.013$, $P \leq 0.016$) variety compared to –Si un-infested plants.In SB infested plants of susceptible variety, the rhythmic lowest and highest peak at 24and 72h post infestation respectively. In resistant variety, the highest peak was observed at 24 and 72h post infestation in +Si+SB treated plants. But in –Si+SB treated plants peak observed only at 72h post infestation. The β-1,3-glucanase activity increased in +Si over –Si treated plants by SB infestation in susceptible variety at 24, 48, 72 and 192 h post infestation ($t \geq 22.013$, $P \leq 0.002$) and in resistant variety at 0, 24, 48 and 72 h post infestation ($t \geq 7.827$, $P \leq 0.016$).In +Si+SB treated plants of susceptible variety, β-1,3-glucanase activity was significantly increased at 24, 48, 72 and 192 h post infestation by 96.4%, 25.1%, 1.3% and 3.9%, and decreased at 0 h post infestation by 9.1% over –Si +SB treated plants. In case of +Si+SB treated resistant plants, β-1,3-glucanase activity was increased at 0, 24, 48 and 72 h post infestation by 6.3%, 21.1%, 8.3% and 10.3%, and decreased at 192 h post infestation by 6.9% as compared to –Si +SB treated plants.

The total phenol content was also much influenced by Si addition, SB infestation, SB infestation and their interactions in both the varieties (Table 1 and 2). As compared to –Si un-infested plants, the total phenol concentration in +Si susceptible (Fig.5C; $t \geq -55.993$, $P \leq 0.002$) and resistant (Fig. 5D; $t \geq -73.135$, $P \leq 0.001$) plants decreased in both the varieties at all sample points. The total phenol concentration in SB infested susceptible plants was much higher in both +Si ($F \geq 7455.907$, $df = 2, 5$, $P \leq 0.0001$) and –Si ($F \geq 817.668$, $df = 2, 5$, $P \leq 0.0001$) plants as compared to uninfected plants. In susceptible plants, the highest peak at 24 and 72 h post infestation where, in resistant plants at 72h post infestation. In between the +Si and –Si treatments, the total phenol concentration increased in +Si treatments due to SB infestation in susceptible variety at all-time points ($t \geq 19.390$, $P \leq 0.003$) while in resistant variety only at 24, 48 and 72h post infestation ($t \geq 13.958$, $P \leq 0.005$) as compared to –Si plants. The phenol concentration in susceptible variety increased over the +Si amendment plants by 9.7%, 35.6%, 26.8%, 25.8% and 6.2% at all-time points. In case of the resistant variety phenol concentration was much increased only at 24, 48 and 72h post infestation by 4.1%, 5.2% and 53.1% respectively over –Si +SB treated plants.

In leaf sheath of both the varieties, soluble protein content was much influenced by Si addition, SB infestation, SB infestation time and between their interactions (Table 1and 2). Without stem borer, soluble protein content in leaf sheath was lower in +Si than –Si plants at all sample points in both susceptible (Fig.5E; $t \geq -43.621$, $P \leq 0.004$) and resistant (Fig.5F; $t=-25.981$, $P =0.001$) varieties. The soluble protein content reduced over infestation time points from 0 hpi to 192 hpi by 36.5% (at 0), 36.1% (at 24), 14.7% (at 48), 13.8% (at 72) and 12.4 (at 192)h post infestation in susceptible variety. But in resistant variety, the most soluble protein content (27.9%) at 48hpi followed by 26.9% (at 0, 72 and 192hpi) and 25.6% at 24h post infestation. In infested +Si susceptible plants, the soluble protein content decreased at 0, 72 and 192h post infestation ($t \geq -43.621$, $P \leq 0.013$) but increased at 24 and 48h post infestation ($t \geq 26.912$, $P \leq 0.001$) as compared to –Si plants. In case of resistant +Si plants, soluble protein content decreased at all-time points except at 48 h post infestation over –Si plants ($t \geq -40.244$, $P \leq 0.007$). The highest peak in +Si+SB plants at 24 and 72h post infestation in susceptible and resistant varieties. The highest soluble protein content in susceptible plants was much increased over –Si plants at 24hpi (90.1%) followed by 48 h post infestation (43.4%) and decreased at 0, 72 and 192 h post infestation by 36.5%, 11.9% and 31.0%. Where in resistant plants, the protein content was much increased as compared to non-silicon amended plants at 48 (7.6%) and 72 h (35.5%) post infestation and decreased at 0, 24 and 192 h post infestation by 26.9%, 27.4% and 17.8%.

Table 1: Three-way analysis of variance for significance (P value) of the effects of silicon amendment to susceptible (Suvra) variety, *Sesamia inferens* infestation and infestation time on physiological parameters. [a] Activities measured, [b] Concentrations measured.

Treatments	SOD[a]	CAT[a]	POD[a]	PPO[a]	PAL[a]	MDA[b]	H_2O_2[b]	Phenol[b]	Soluble Protein[b]	β-1,3- Glucanase[a]
Si amendment (A)	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Infestation (B)	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Infestation time (C)	0.7222	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	0.0798	*P*<0.0001	*P*<0.0001	*P*<0.0001
A × B	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
A × C	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
B × C	0.1544	*P*<0.0001	*P*<0.0001	0.0057	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
A × B × C	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001

Table 2: Three-way analysis of variance for significance (P value) of the effects of silicon amendment to resistant (HR-379)variety, *Sesamiainferens* infestation and infestation time on physiological parameters.[a] Activities measured,[b] Concentrations measured.

Treatments	SOD[a]	CAT[a]	POD[a]	PPO[a]	PAL[a]	MDA[b]	H_2O_2[b]	Phenol[b]	Soluble Protein[b]	β-1,3- Glucanase[a]
Si amendment (A)	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Infestation (B)	0.7068	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
Infestation time (C)	*P*<0.0001	0.6037	0.0014	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
A × B	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
A × C	*P*<0.0001	0.0003	0.508	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
B × C	0.9168	0.0107	*P*<0.0001	0.0715	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001
A × B × C	*P*<0.0001	0.0002	0.0009	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001

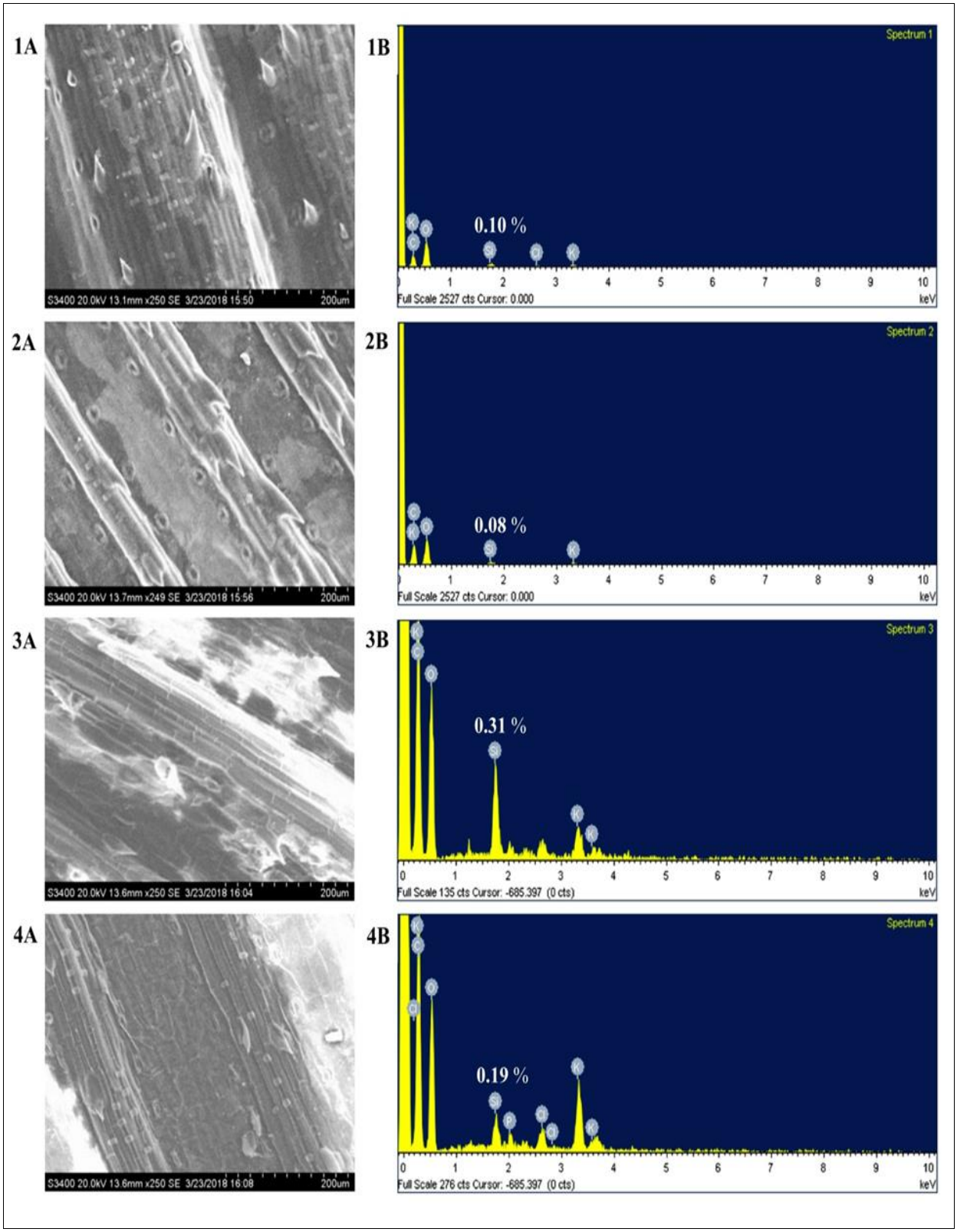

Fig 1: SEM analysis of silicon deposition in leaf sheath surface of the variety 'Suvra' after 24hpi showing silica cells and deposition in different treatment. 1A & B: without Si addition and with stem borer (SB) infestation (-Si+SB); 2A & B: without Si addition and SB infestation (-Si-SB); 3A-B: with Si addition and SB infestation (+Si+SB) and 4A & B: with Si addition and without SB infestation (+Si-SB). (250x,Bar = 200 µm.). Energy dispersive X-ray (EDX) mapping of silicon (yellow colour with percent) (1B-4B). Values in percent are means ± SD from 3 biological replicates.

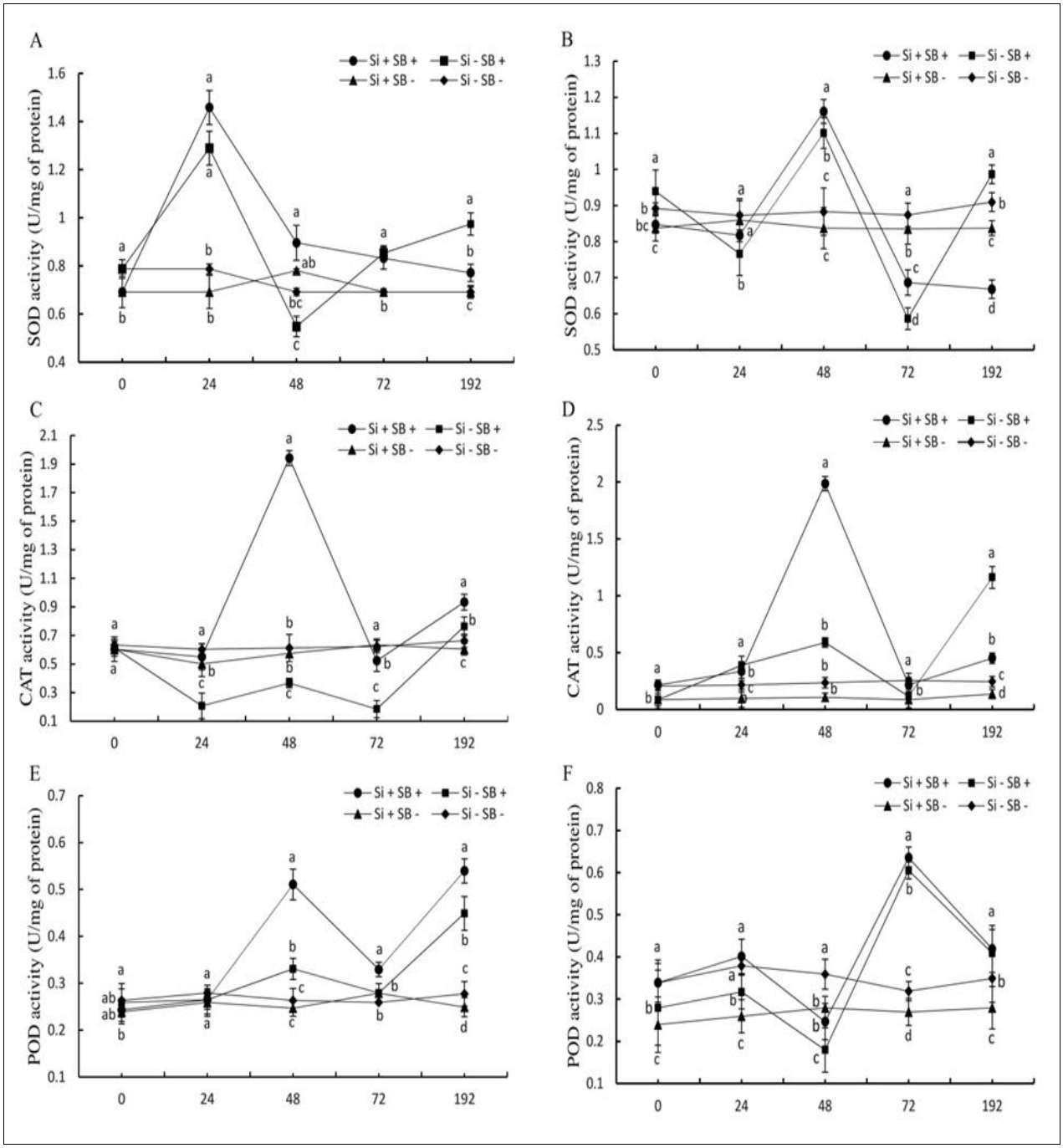

Fig 2: Effect of silicon amendment and stem borer infestation on antioxidant enzyme activities in both susceptible and resistance varieties. Susceptible variety: (A) Superoxide dismutase, SOD. (C) Catalase, CAT. (E) Peroxidase, POD Resistant variety: (B) Superoxide dismutase, SOD. (D) Catalase, CAT. (F) Peroxidase, POD. +Si = silicon amendment at 1mM Si/L nutrient solution, −Si = no silicon amendment. +SB = infestation of *S. inferens* larvae,–LF = no infestation. Values are means ± SD from 3 biological replicates. In each panel, the means labelled by different letters at a certain time point post *S. inferens* infestation are significantly different at $P < 0.05$ according to Tukey's multiple range tests.

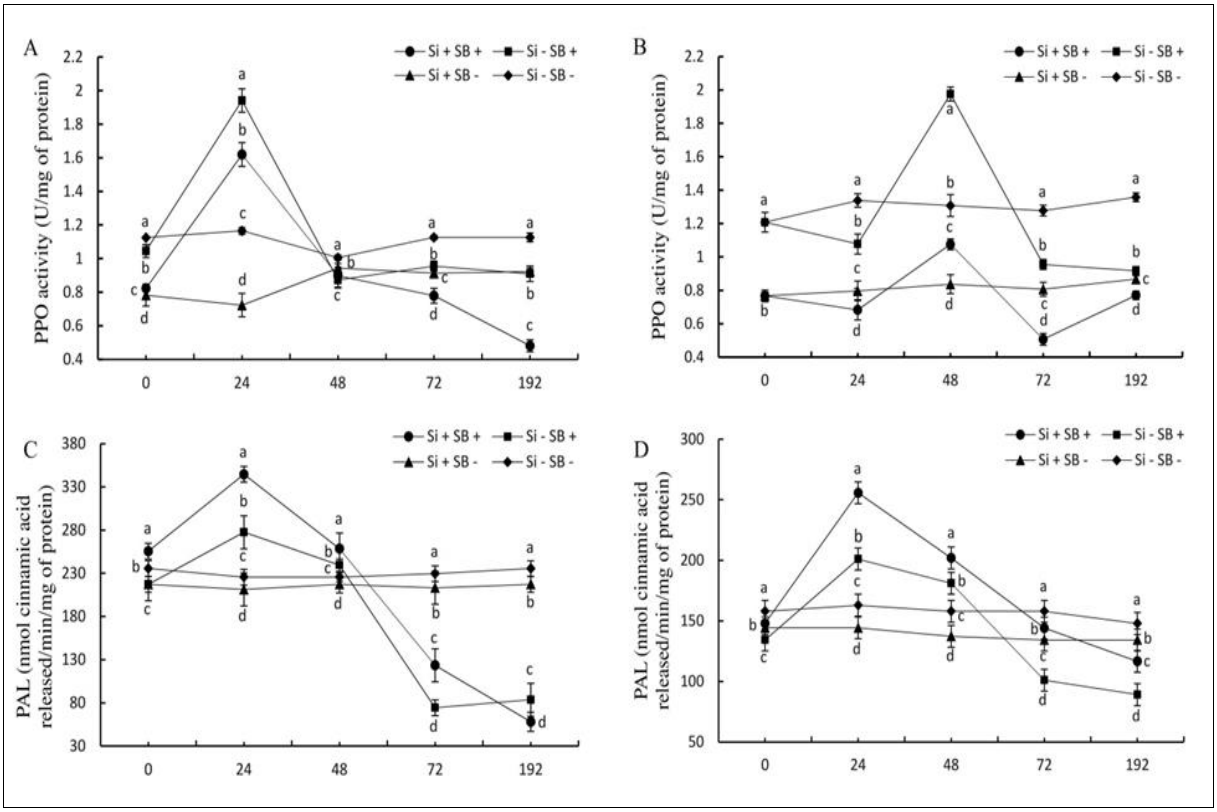

Fig 3: Enzyme activity for production of secondary metabolites in susceptible and resistance variety of ragi leaf sheath in response to silicon amendment and stem borer infestation. Susceptible variety: (A) Polyphenol oxidase, PPO. (C) Pheny-lalanine ammonia-lyase, PAL. Resistant variety: (B) Polyphenol oxidase, PPO. (D) Pheny-lalanine ammonia-lyase, PAL. +Si = silicon amendment at 1mM Si/L nutrient solution, −Si = no silicon amendment. +SB = infestation of *S. inferens* larvae, –LF = no infestation. Values are means ± SD from 3 biological replicates. In each panel, the means labelled by different letters at a certain time point post *S.inferens* infestation are significantly different at $P < 0.05$ according to Tukey's multiple range tests.

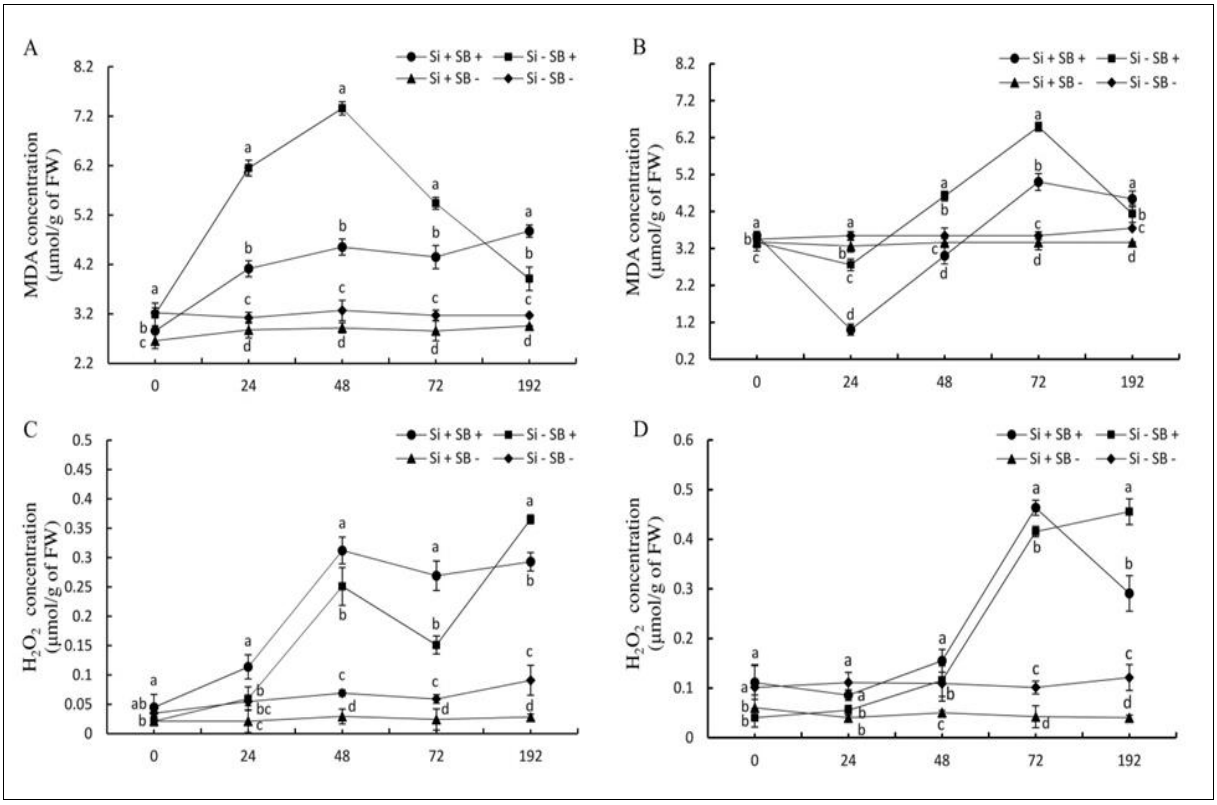

Fig 4: Concentrations of malondialdehyde and H_2O_2 in ragi leaf sheaths in response to Si amendment and *S. inferens* infestation. Susceptible variety: (A) Malondialdehyde, MDA. (C) H_2O_2. Resistant variety: (B) Malondialdehyde, MDA. (D) H_2O_2. +Si = silicon amendment at 1mM Si/L nutrient solution, −Si = no silicon amendment. +SB = infestation of *S. inferens* larvae, –LF = no infestation. Values are means ± SD from 3 biological replicates. In each panel, the means labelled by different letters at a certain time point post *S.inferens* infestation are significantly different at $P < 0.05$ according to Tukey's multiple range tests.

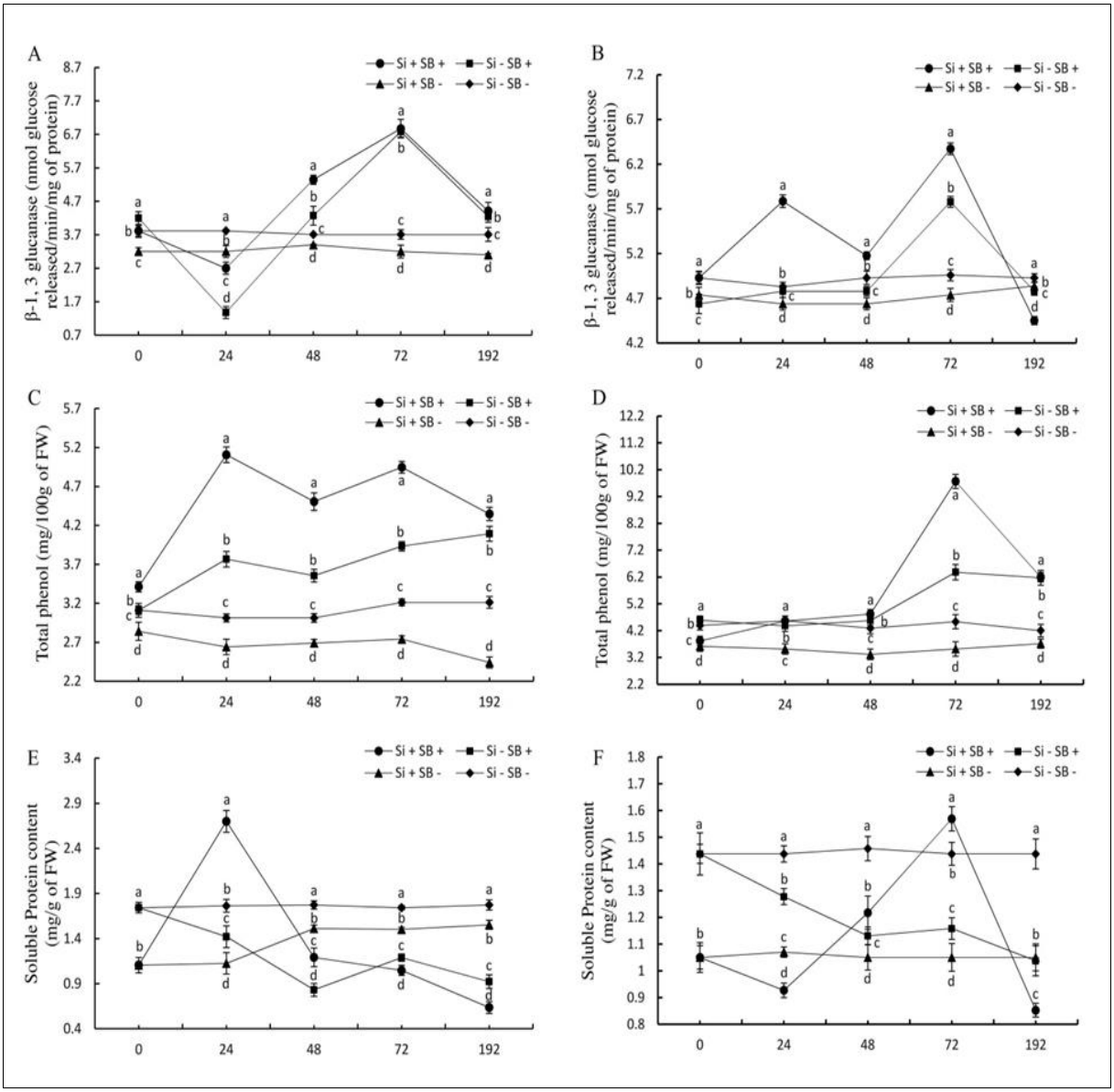

Fig 5: Effects of silicon amendment and stem borer infestation on activity of β-1,3-glucanase, total phenol and soluble protein content in ragi leaf sheaths. Susceptible variety: (A) β-1,3-glucanase (C) total phenol (E) Soluble protein content. Resistant variety: (B) β-1,3-glucanase (D) total phenol (F) Soluble protein content. +Si = silicon amendment at 1mM Si/L nutrient solution, −Si = no silicon amendment. +SB = infestation of *S. inferens*larvae, –LF = no infestation. Values are means ± SD from 3 biological replicates. In each panel, the means labelled by different letters at a certain time point post *S. inferens* infestation are significantly different at $P < 0.05$ according to Tukey's multiple range tests.

Discussion

Major cereal crops are known for its higher silicon accumulation ability including rice through an active process (Hodson *et al*., 2005; Ma *et al*., 2006) [15, 24]. Despite that silicon was not a part of an essential element but has significant role in both biotic and abiotic stresses. One of the major mechanisms of Si-mediated plant resistance was the physical barrier through silicon deposition in epidemic cells to prevent the entry of invading insect, pest and pathogens. The present study also confirms that the physical barrier mechanism in ragi plants by silicification of leaf sheaths which clearly indicates decrease the tunnel length, bore diameter, and typical dead heart symptoms in silicon amended ragi plants. Plant resistance to insect and pathogen due to silicon deposition in plant as reported in asiatic rice borer (*C. suppressalis*) (Hou and Han,2010) [14], the sugarcane borer (*D. saccharalis*) (Sidhu *et al*. 2013) [43], African stem borer (*E. saccharina*, Walker) (Keeping and Meyer, 2002;2006; Kvedaras and Keeping, 2007a,b) [16, 17, 19, 20] and for other herbivores, such as rice leaf folder (*C. medinalishas*) (Ye *et al*. 2013; Han *et al*. 2015; Han *et al*. 2016) [51, 12, 13], African armyworm (*S. exempta*) (Massey *et al*., 2006) [25], the brown plant hopper (*N. lugens*) and the desert locust (*S. gregaria*) (Massey *et al*., 2006) [25]. However, a physical barrier mechanism alone cannot explain the entire role of silicon in suppressing insect pests including stem borer (Massey and Hartley, 2009) [26]. But, it has a vital role in defense-associated signaling pathways which regulates a range of physiological activities in during stress (Ye *et al*. 2013; Van Bockhaven *et al*., 2013) [51, 48]. ROS are the components of plant defense responses which activates the signal transduction processes in response to pathogen and herbivore attacks. It is also stated that the excessive levels of ROS can cause significant damage to cell structures and plants in counter produces an antioxidant enzymes (SOD, POD and CAT), a first defense mechanism to protect themselves from excessive levels of ROS (Foyer *et al*. 1994) [8]. SOD catalyzes superoxide anion free radicals into H_2O_2 by POD and CAT. Silicon plays a crucial role in plant tolerance by altering activity of antioxidant enzymes, cation binding capacity of the

cell walls and endogenous the plant hormone level in various stresses (Sivanesan and Park, 2014) [41]. In the present study, the activities of SOD, CAT and POD increased as well as decreased to certain time points in response to SB infestation. The results explain that SB infeststation increases the uptake of silicon and amplify the activities of antioxidative enzymes to detoxify excessive ROS level in Si amendment plants, which was similar with earlier reports in *Arabidopsis*, rice and perennial ryegrass plants in their responses to Si application and disease infection (Fauteux, 2006;Rahman *et al*. 2015) [9, 34]. H_2O_2 stimulates an array of cascade for expression of defense genes, preventing the plants being hurt by the attack of pathogens and herbivores (Torres, 2010) [47]. The result indicates that the H_2O_2 concentration increased with Si amended than in without Si infested plants which might give to the differential expression of defense genes between with Si and without Si amendment plants. H_2O_2 has potential for providing resistance to herbivores through a direct effect on insect physiology, as in the case, as reported in European corn borer, *Ostrinia nubilalis* (Ramputh *et al*.,2002) [35]. It concluded that the higher H_2O_2concentration in the with Si infested plants, might partly explain the reduced tunnel length, bore diameter, and dead heart symptoms in Si amended ragi plant. ROS involved in high levels of lipid peroxidation by increasing the MDA concentration, which recognized biomarker for the degree of cell membrane damage. In the present study it observed that MDA content in leaf sheath increased in response to SB infestation alone, but the increases were remarkable in without Si and with SB plants in contrast to with silicon and stem borer amended plants. The results also suggests that with Si amendment plants might be less damaged compared without Si plants and hence functions to less MDA accumulation in infested plants, and thus give protection to the stressed plants.

Many plant secondary metabolic compounds have a dominant role in defense against herbivores, pests and pathogens which are resistance to biotic stress (Bennett and Wallsgrove, 1994) [3]. Both PAL and PPO enzyme activities involved in biosynthesis of phytoalexins, phenols, and lignins that can restrict the development of herbivorous insects (Subbarao and Towers, 1990) [45]. The present findings revealed that PPO and PAL activities triggered by stem borer infestation, but a rhythmic up and down activity pattern was observed in case of PPO at the time points particularly in resistant infested plants. Generally, the PPO activity increased in SB infestation alone, but the increases were prominent in without Si and with SB plants as compared with Si and SB plant. It indicates that Si alters PAL and PPO activities, and confers increased resistance to herbivores. Ye *et al.* (2013) [51] noted that POD and PPO activities did not respond to Si addition, but generally increased more in +Si+LF plants in comparison to -Si+LF plants. Gomes *et al*. (2005) [11] reported a different pattern for the wheat aphid system, where Si addition, infestation with aphids and their interaction all much enhanced PPO activity, while Si addition did not affect the PAL activity in wheat plants. In the present study, SB infestation triggers the β-13-glucanase activity level in with Si amended plants than in without Si plants. Chérif *et al*. (1994) [6] reported the activity of β-1,3glucanase increased in silicon amended cucumber plants due to pathogen infection. The activities of β-1,3-glucanase increased in potato plants against the disease caused by *Rhizoctonia solani* AG-3 (Wolski *et al*., 2006) [49]. Therefore, it is clear that the enhanced activities of β-1,3-glucanase associated with Si amendment may have benefited Si-mediated ragi plants in their resistance to SB infestation.

Phenolics are biologically active secondary metabolites directly affect the insect growth and feeding. There are several reports showing the induction of phenols in plants response to insect attack (Sharma *et al*., 2009) [40]. The results revealed that the total phenol content in +Si plants increases much more than –Si plants in infested susceptible and resistant varieties which indicates that phenolics have a role in the plant defense (Avdiushko *et al*., 1993; Sudhakar *et al*., 2001) [2, 44]. Plant soluble protein was the main source of amino acids as well as an indicator of food quality for herbivores. Interestingly, in the present study, silicon amendment alone decreases soluble protein content in Si amendment plants over without Si amendment plants which indicate low quality food for stem borer. Reduce the level of soluble protein content in +Si plants than in -Si plants after certain time points due to SB infestation. The result confirmed that the herbivory infestation results in reduced soluble protein content in host plants due to loss photosynthetic capacity by pest damage and vigorous synthesis of defensive enzymes (Chen *et al*., 2009; Padmavathi *et al*., 2013; Singh *et al*., 2013) [5, 30, 42].

In conclusion, the present study shows that Si amendment helps direct defense through increased physical resistance to stem borer infestation by intensified silicification in ragi leaf sheath which decreases tunnel length, bore diameter, and typical dead heart symptoms. Silicon amendment much influenced the activities of antioxidant enzymes, syntheses of β-1,3-glucanase, total Phenol and soluble protein content in the stem borer infested ragi plants which signifies the indirect chemical defense mechanism. The present findings also prove that Si amendment, through interactions with SB infestation, plays a major role from a physical barrier to priming intensified plant defense responses for enhanced plant resistance to herbivores which further leads to the understanding of mechanisms for enhanced plant resistance to pink stem borer and/or herbivores. Secondary metabolic compounds such as PPO, soluble protein content, and total phenol have greater role in response to both Si amendment and stem borer infestation. These findings have a potential alternative for stem borer management that is of important agricultural and ecological implications.

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