Research Article

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Evidence for rice tolerance to *Tibraca limbativentris* (Hemiptera: Pentatomidae)

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Abstract

BACKGROUND: The rice stalk stink bug *Tibraca limbativentris* (Hemiptera: Pentatomidae) is one of the most important rice pests in Brazil. The search for cultivars that tolerate insect injury is necessary to complement other less aggressive methods of pest suppression. The combination of integrated pest management tactics will reduce insecticide applications and improve the safety of food production. Here, we tested the tolerance response of Xingu, Canela de Ferro and Primavera rice genotypes in glasshouse experiments. In addition, we measured tolerance expressed in a variety of physiological responses, including gas exchange rates, leaf chlorophyll content and reactive oxygen species (ROS) detoxification.

RESULTS: The results showed that the tolerance of the Primavera genotype to rice stalk stink bug damage was higher, due to (a) a lower reduction of photosynthetic activity, (41% reduction only 96 h after infestation) compared to Xingu and Canela de Ferro (56 and 65% reduction at 24 and 48 h after infestation, respectively); (b) the capacity to maintain the chlorophyll content after infestation, while Xingu and Canela de Ferro reduced their chlorophyll content to 20% and 25% at 72 and 48 h after infestation, respectively; (c) the antioxidative defense system being activated in the first 12 h after infestation, in which superoxide dismutase (SOD) showed an increase of 61% in its activity, and (d) the maintenance of its grain yield, number of panicles per plant, number of filled grains, and spikelets sterility.

CONCLUSION: Rice genotypes tolerant to herbivory can be identified by measuring the effect of injury and the plant's physiological response by evaluating attributes such as grain yield and its components, gas exchange, chlorophyll content and ROS detoxification. Therefore, the use of rice genotypes tolerant to stalk stink bugs as a component of integrated pest management has the potential to reduce upland rice yield loss.

Keywords: agronomic traits; antioxidant system enzymes; chlorophyll content; gas exchange; rice stalk stink bug

1 INTRODUCTION

Host plant resistance (HPR) is one of the most effective and sustainable pest control tactics and is non-polluting and accessible to farmers.[1] Three categories of HPR have been proposed: antixenosis, antibiosis and tolerance. In antibiosis, the plant negatively affects insect biology/physiology and may even cause insect death. In antixenosis, the plant has an adverse effect on insect behavior, making it an unsuitable host for food, oviposition or shelter. In tolerance, the plant presents the ability to recover from or withstand injury by maintaining normal vital functions through the activation of biochemical-molecular defense mechanisms, even under stressful conditions.[2,3] In addition, tolerance characteristics have no effect on the pest such that consequent selection of resistant pest populations is unlikely.[2,4] However, antibiosis and antixenosis can exert pest selection pressures, which may result in pests overcoming plant resistance, as they interfere with insect biology/physiology.[5]

When the plant's tolerance involves compensatory traits for itself, the plant is able to harbor large numbers of herbivores without interfering with the insect pest's physiology or behavior.[2] According to Mitchell *et al.*,[6] plant tolerance occurs when plant traits reduce the negative effects of herbivore damage on crop yield. Plant tolerance to insects is a genetic condition expressed through various biochemical and physiological events related to primary processes such as photosynthetic activity, growth and detoxification.[2] Studies have shown reduced photosynthetic rates and chlorophyll contents in susceptible plants compared to insect-tolerant plants.[7–10] According to Buffon *et al.*,[11] the photosynthetic rate in rice variety IRGA 423 (tolerant) was less affected by *Schizotetranychus oryzae* (Acari: Tetranychidae) infestation than it was in plants of the variety Puitá INTA-CL (susceptible). The wheat-tolerant variety (Halt) infested with *Diuraphis noxia* (Hemiptera: Aphididae) showed a photosynthetic rate similar to that of the control plants (without infestation), while the susceptible variety (TAM 107) showed a reduction in the photosynthesis rate.[7]

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In response to initial insect feeding, ROS (reactive oxygen species) such as superoxide radicals ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), singlet oxygen ($O^{\cdot}$), and hydroxyl radicals ($OH^{\cdot}$) have been recognized as central early signals, integrating environmental information and regulating stress tolerance.[9,11,13] Normally, plants display exceptional redox control, using ROS and antioxidants, such as ascorbate and glutathione, to regulate numerous aspects of their biology, including metabolism, growth, development and gene expression patterns.[13–16] Under normal conditions, ROS are rapidly detoxified, and cellular redox homeostasis is governed by the presence of enzymes, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (POX), glutathione peroxidase, peroxiredoxin, thioredoxin, glutaredoxin, glutathione reductase and dehydroascorbate reductase,[17] and large pools of antioxidants that remove and buffer against oxidants.[12,18]

However, an oxidative burst in response to environmental stresses may lead to the generation of excessive ROS.[19] In this scenario, if the excessive accumulation of ROS is not efficiently removed, it can become toxic to plant cells, rapidly oxidizing and damaging cellular components, and ultimately lead to cell death.[2] Under proper developmental conditions, cell ROS production is low (240 mmol L^{-1} s^{-1} O_2^- and the steady-state level of 0.5 mmol L^{-1} s^{-1} H_2O_2 in chloroplasts), while many stresses that alter cellular homeostasis accentuate ROS production (240–720 mmol L^{-1} s^{-1} O_2^- and 5–15 mmol L^{-1} H_2O_2).[20] When injured by insects, tolerant plants increase the activity of antioxidant system enzymes as a cell detoxification mechanism.[2,21–23] In tolerant rice genotypes, an increase in POX and polyphenol oxidase activity was observed when *Nilaparvata lugens* (Hemiptera: Delphacidae) infested plants.[24] In tobacco, tolerance to damage caused by whiteflies (Hemiptera: Aleyrodidae) can be explained by increased CAT activity.[25]

The rice stalk stink bug *Tibraca limbativentris* Stål, 1860 (Hemiptera: Pentatomidae) is recognized as the major rice pest in Brazil and is also important throughout South and Central America.[26–28] This species also presents potential as an invasive pest in the United States.[29] The damage caused by rice stalk stink bugs can occur during the vegetative phase, which leads to a 'dead heart', or in the reproductive phase, which leads to a 'white-head'.[30] Under high infestation conditions, the yield loss caused by the pest can reach up to 90%.[31]

Plant perception of hemipteran pests appears to occur within a short time frame <1 h, with some changes observed within an even shorter interval.[16,32] Reaction cascades impacted by a number of cell wall-anchored proteins include changes in intercellular calcium content and the production of superoxide and related ROS. Some of these events are likely part of the innate immunity of plants to pests and/or pathogens.[18] Piercing-sucking insects subsequently trigger more specific responses because the removal of the phloem and xylem contents disturbs both the water and nutrient balance in the plant and effectively modulates chloroplast functions.[2]

The most common method used to control rice stalk stink bugs is chemical insecticide applications.[33] However, this method can be inefficient due to inadequate contact between the insect and the insecticide because the insect usually settles at the base of the rice stem to feed.[34] The use of only insecticides to control stink bugs can lead to undesirable effects on the environment, non-target organisms and humans, as well as favoring the selection of insects resistant to insecticides.[33,35] Therefore, it is necessary to incorporate more efficient and less environmentally disruptive control methods compatible with integrated pest management (IPM).[6]

Rice (*Oryza sativa* L.) has an evolutionary peculiarity of being semiaquatic, and flooded rice paddies have become the major form of rice cultivation; growth in irrigated and rainfed lowland conditions occurs in 75% and 19% of the global production area, respectively. Upland rice represents only 4% of global rice production and is grown on less than 9% of Asia's total rice acreage, with 46% in Latin America and 47% in West Africa.[36] As rice is an essential crop for food security in more than half of the world's population,[1] it is of utmost importance to assess the impact of herbivory on agronomic and physiological attributes such as grain yield and its components, gas exchange, chlorophyll content and antioxidant systems. However, plant tolerance to insects has been poorly studied due to barriers in the determination of defense action mechanisms, as well as factors that hamper high-precision phenotyping methods for large-scale screening.[6,37]

2 MATERIALS AND METHODS

To characterize the tolerance of upland rice to stalk stink bugs, two experiments were carried out in duplicate at Embrapa Arroz e Feijão. One experiment sought to evaluate the agronomic performance of plants attacked by this insect, and the other experiment aimed to evaluate the physiological responses of injured plants. We use three rice genotypes (*O. sativa* L.) that are known for their different degrees of resistance to rice stalk stink bug. The Xingu genotype, BGA 014016, a commercial cultivar, is susceptible to rice stalk stink bug.[38] The Canela de Ferro genotype, BGA 011523, landrace, and Primavera, BGA 008070, are both commercial cultivars that present agronomic traits such as high grain yield and consumer acceptance; both genotypes are resistant to the rice stalk stink bug by antibiosis and/or antixenosis.[38] The three genotypes belong to the gene bank of Embrapa Rice and Beans and constitute genetic diversity within the upland rice breeding program.

2.1 Insects rearing

For oviposition, *T. limbativentris* adults were field-collected (Embrapa Rice and Beans experimental area), kept in cages and fed rice plants (cultivar BR IRGA 409) in a glasshouse. The eggs were collected and transferred to a Gerbox-type plastic container (11 cm × 11 cm × 3.5 cm, Adria Laboratórios, Londrina, Paraná, Brazil) lined with a moistened paper towel and kept in the laboratory (temperature 25 ± 2 °C, relative humidity 70 ± 10% and photoperiod 14 h) until nymphs hatched; nymphs remained under these conditions until the second instar.

After this phase, the nymphs were transferred to rice plants (the same variety) at age 45 to 50 days and covered with voile mesh cages until the adult stage for oviposition. The plants were inspected every 2 days, with dead insects and ovipositions being removed.

2.2 Agronomic attributes

2.2.1 Grain yield and production components

The upland rice plants were obtained using the same methodology as in the previous experiment. At 10 days after emergence (DAE), the seedlings were transplanted to 8 L plastic pots containing commercial soil substrate (BIOPLANT - Nova Ponte, MG, Brazil). Three plants were transplanted into each plastic pot, with each plastic pot being considered one replicate, totaling six replicates in a completely randomized design. The plants received the recommended fertilization for rice cultivation[39] and were irrigated periodically according to their water requirements. The

plants were kept in a glasshouse with environmental conditions monitored by a datalogger (Hobo® U12-012, Onset Computer Corp. Ltd, Cape Cod, MA, USA). Temperatures ranged from 16.5 to 32.7 °C (22.8 °C average), and relative humidity ranged from 20.7% to 85.5% (45.2% average).

At 48 DAE, the plants of the three genotypes were infested with two pairs of adult rice stalk stink bugs (15 days) for a period of 8 days. The plants were covered with a screen cage (50 mesh). After infestation, all plants were subjected to insecticide application (tiamethoxan + λ-cyhalothrin, Engeo Pleno®) to ensure no insect damage to the plants.

Harvesting was performed manually after physiological maturity of the grains. The agronomic traits evaluated were grain yield (GY in g plant^{-1}, which means the total mass of grains, in grams, obtained for three plants per pot) and its components, such as the number of panicles per plant (NPP), number of filled grains (NFG, filled grains average in six panicles per column), and number of empty grains (NEG, empty grains average in six panicles per column). Spikelet sterility was estimated as SS = (NEG × 100)/TG, where SS is the spikelet sterility, NEG the number of empty grains, and TG the total number of grains.

2.3 Physiological traits

2.3.1 Plant growth

Seeds of the three genotypes were sown in a Styrofoam tray (128 cells) filled with commercial soil substrate. At 10 DAE, the seedlings were transplanted to 8 L plastic pots. Each plastic pot containing two plants was considered one replicate, totaling eight replicates in a completely randomized design. The plants were kept in a glasshouse with environmental conditions monitored by a datalogger (Hobo® U12-012, Onset Computer Corp. Ltd). The temperatures ranged from 20.5 to 35.7 °C (26.7 °C average), and the relative humidity ranged from 43.7% to 94.2% (77.9% average). The plants received the recommended fertilization for rice cultivation[38] and were irrigated periodically according to their water requirements.

2.3.2 Plant infestation

At 49 DAE, each of the two rice plants in each plastic pot was covered with a voile mesh cage, one of which was infested with an adult rice stalk stink bug female (12 days, starved for 8 h before infestation), while the other plant was left uninfested (control treatment). The insect was placed in contact with the rice stalk for 12, 24, 48, 72 and 96 h. Each of the time periods constituted separate treatments, independent of each other, taking care not to damage the plant and interfere with the physiological analysis. At the end of each time, the insect was removed, and physiological evaluations were performed.

2.3.3 Gas exchange

The carbon dioxide (CO_2) assimilation rate (A, in μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, in mmol H_2O m^{-2} s^{-1}), stomatal conductance (g_s, in mol H_2O m^{-2} s^{-1}), and internal CO_2 concentration (CI, in μmol mol^{-1}) were determined on the middle third of the first fully expanded leaf (top to base) of upland rice plants. Measurements at 12, 24, 48, 72 and 96 h after rice stalk stink bug infestation were performed using a portable infrared gas analyzer (LCpro+, ADC BioScientific, Hoddesdon, UK). The equipment was set to use temperature and concentrations of 370 to 400 mol mol^{-1} CO_2 in air, which is the reference condition used in the IRGA photosynthesis chamber. The photon flux density photosynthetically active (PPFD) was 1200 μmol [quanta] m^{-2} s^{-1} in the chamber with artificial light, where the leaf to be analyzed was placed. The minimum equilibration time set for performing the reading was 2 min. Two readings per plant, between 8:00 and 10:30 h, were performed at each repetition per treatment.

2.3.4 Chlorophyll content

Leaf chlorophyll content measurements were performed at 12, 24, 48, 72 and 96 h after rice stalk stink bug infestation of the rice plants. Measurements were indirectly performed by a SPAD-502 (Konica Minolta Sensing, Inc., Tecnal Laboratory Equipment, Piracicaba, Brazil)[40] portable meter using the optical density difference of two wavelength regions (650 nm and 940 nm). Ten readings per plant, between 8:00 and 10:30 h, were performed during each repetition per treatment.

2.3.5 Superoxide dismutase, catalase and peroxidase activity

2.3.5.1. Crude extract. The first fully expanded leaf (top to base) was collected at 12, 24, 48, 72 and 96 h after infestation of the rice plants to determine its enzymatic activity. After collection, the leaves were placed in 50 mL Falcon centrifuge tubes and kept in liquid nitrogen. Then, they were subjected to freeze drying at −80 °C with lyophilization, and analytical processing was subsequently performed. To obtain the leaf extract, 60 mg of lyophilized leaf, 20 mL of 100 mmol L^{-1} potassium phosphate buffer solution, pH 7.8, containing 0.1 mmol L^{-1} EDTA, 0.44% (*p/v*) polyvinylpyrrolidone K-40 (PVP) and 0.5% (*v/v*) Triton X-100 was used. Then, the mixture was homogenized for 3 min in a vortex shaker, and the homogenate obtained was centrifuged at 11 000 rpm for 40 min at 4 °C. The supernatant, referred to as the crude leaf extract, was fractionated into 1000 μL aliquots and stored at −20 °C.[41]

2.3.5.2. Superoxide dismutase specific activity. Briefly, 50 μL aliquots of the crude rice leaf extract were used to determine SOD activity,[42] with modifications. The method is based on the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). According to Corte *et al.*[43] one unit of SOD is defined as the amount of enzyme that causes a 50% decrease in SOD-inhibitable NBT reduction. The results were expressed as SOD units (UN SOD mg^{-1} protein), defined as the ratio of SOD activity to the total amount of soluble protein contained in the crude extract, with the total soluble protein content of the crude leaf extract being determined according to Bradford.[44]

2.3.5.3. Catalase specific activity. Briefly, 50 μL aliquots of the crude rice leaf extract were used to determine CAT activity,[45] with minor modifications. The catalase kinetic assay was determined by the disappearance of H_2O_2 as measured by ultraviolet spectrophotometry and expressed as mmol reduced H_2O_2 min^{-1}. To determine the specific activity (mmol reduced H_2O_2 min^{-1} mg^{-1} protein), defined as the ratio of CAT activity to the total amount of soluble protein contained in the crude extract, the total soluble protein content in the crude leaf extract was determined according to Bradford.[44]

2.3.5.4. Peroxidase specific activity. Brifly, 50 μL aliquots of the crude rice leaf extract were added to the reaction medium consisting of 0.1 mol L^{-1} acetate buffer pH 5.0, 0.3% H_2O_2 and 1.0 mmol L^{-1} 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS).[46,47] The POX activity results are expressed in mmol ABTS* s^{-1}. To determine the specific activity (mmol ABTS* s^{-1} mg^{-1}),

defined as the ratio of POX activity to the total amount of soluble protein contained in the crude extract, the total soluble protein content in the crude leaf extract was determined according to Bradford.[44]

2.3.5.5. Total soluble protein. The total soluble protein concentration for all samples was determined by the Bradford method[43] using bovine serum albumin (BSA) from a stock 1 mg mL^{-1} BSA standard solution. The total soluble protein content in the leaf extract was calculated by reference to the calibration graph plotted from the results obtained with the diluted standard solutions containing 0, 25, 75, 125, 175, 225, and 300 μg mL^{-1} BSA.

2.4 Statistical analysis

Data were analyzed for normality by the Kolmogorov–Smirnov test and homoscedasticity by the Bartlett test. Then, the means that assumed a normal distribution were submitted to joint analysis of variance for replicates, and Student's *t*-test ($P < 0.05$) was used to verify if there were differences between the injured and uninjured plants. When the assumptions were not met, the means were submitted to joint analysis of deviance for replicates (ANODEV) ($P < 0.05$), fitting Poisson generalized linear models (GLMs) for the variables NPP and NFG and a binomial for the variable SS. All analyses were performed using R version 4.0.1 (www.rproject.org).

3 RESULTS

3.1 Agronomic attributes

The GY and NFG of the injured plants of the Xingu and Canela de Ferro genotypes showed significant differences relative to uninjured plants (Table 1). For the NPP, no significant differences were observed between plants injured by rice stalk stink bugs and uninjured plants in any of the evaluated genotypes.

In the Xingu and Canela de Ferro genotypes, NFG was approximately 27% lower in injured plants than in uninjured plants. In the Primavera genotype, no reduction in NFG between injured and uninjured plants was observed. In the Xingu and Canela de Ferro genotypes, the reduction in GY in the injured plants was 29% and 24%, respectively. In the Primavera genotype, no significant difference in GY between injured and uninjured plants was observed.

In the Xingu and Canela de Ferro genotypes, the increase in SS in injured plants was approximately 112% compared to uninjured plants. In the Primavera genotype, no increase in SS between injured and uninjured plants was observed.

3.2 Physiological traits

In the three genotypes evaluated, the plants infested with rice stalk stink bugs showed a significant reduction in *A*, *E* and g_s compared with the uninjured plants (Figs 1, 2 and 3). On average, the net photosynthesis value was approximately 12.5 μmol CO_2 m^{-2} s^{-1} for uninjured plants of the Xingu, Canela de Ferro and Primavera genotypes during 96 h of evaluation. Transpiration and stomatal conductance values, on average, were approximately 3.5 mmol H_2O m^{-2} s^{-1} and 0.23 mol H_2O m^{-2} s^{-1}, respectively. Although within the accepted standards for upland rice genotypes,[48] these values are considered low for modern cultivars.

For the injured plants, Xingu presented photosynthetic activity reduction 24 h after infestation. Over the subsequent hours (24 to 96 h after insect contact with the rice plants), the average reduction was approximately 56% ($t_{1,30} = -5.66$, $P = 0.0005$), 26% ($t_{1,30} = -2.71$, $P = 0.016$) and 40% ($t_{1,30} = 4.87$, $P < 0.001$), respectively. In the Canela de Ferro genotype, the reductions in *A*, *E* and g_s were even more pronounced (65%, 36% and 42%, respectively), beginning at 48 ($t_{1,30} = 4.78$ $P = 0.0002$), 72 ($t_{1,30} = 2.56$, $P = 0.022$) and 96 h ($t_{1,30} = -2.23$, $P = 0.042$) after infestation, respectively. In the Primavera genotype, the reduction in *A* (41%) ($t_{1,30} = -2.82$, $P = 0.0013$), *E* (36%) ($t_{1,30} = -5.48$ $P = <0.001$) and g_s (40%) ($t_{1,30} = -4.36$, $P < 0.001$) occurred within 96 h after infestation. In relation to CI, plants of the Xingu and Canela de Ferro genotypes injured by rice stalk stink bugs showed significant increases compared with uninjured plants (Fig. 4). An increase of 18% in CI in both the Xingu ($t_{1,30} = 4.09$, $P = 0.001$) and Canela de Ferro ($t_{1,30} = 2.89$, $P = 0.011$) genotypes was observed starting 72 h after infestation. However, the Primavera genotype presented no significant increase in CI compared to uninjured plants.

Table 1. Individual effect of *Tibraca limbativentris* infestation on grain yield and its components of rice genotypes

Variety	Uninjured	Injured	*P*-Value	Percent	$t_{(1,22)}$
Grain yield (GY) (g plant^{-1})					
Xingu	23.60 ± 0.001	16.82 ± 0.002	0.0319	−34.4	2.52
Canela de Ferro	25.27 ± 0.001	19.29 ± 0.001	0.0078	−24.7	3.36
Primavera	19.26 ± 0.002	19.44 ± 0.002	0.9602	0	0.05
Number of panicles plant (NPP)					
Xingu	7 ± 0.001	6 ± 0.001	0.4967	−14.3	0.46
Canela de Ferro	7 ± 0.0006	5.16 ± 0.0004	0.1971	−26.3	1.66
Primavera	6.83 ± 0.7	5.83 ± 0.6	0.4911	−14.7	0.47
Number of filled grains (NFG)					
Xingu	597.1 ± 48.3	440 ± 65.5	< 0.001	−26.4	13.6
Canela de Ferro	825.5 ± 53.3	605.1 ± 26.1	< 0.001	−26.7	204.4
Primavera	719.1 ± 42.1	794.5 ± 76.0	< 0.001	0	22.5
Spikelet sterility (SS) (%)					
Xingu	5.03 ± 2.86	10.63 ± 1.28	< 0.001	+111	101.75
Canela de Ferro	7.63 ± 3.35	16.18 ± 1.25	< 0.001	+112	197.2
Primavera	9.53 ± 1.14	8.09 ± 1.01	0.0147	0	5.94

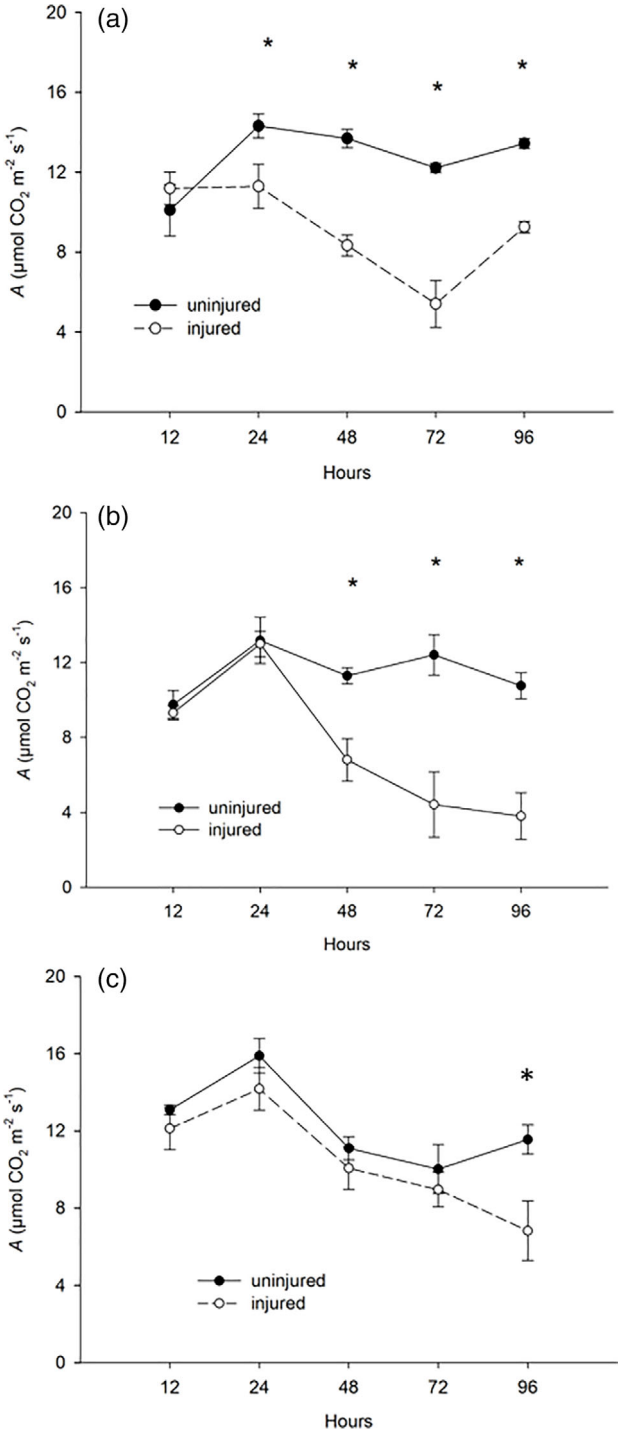

Figure 1. Assimilation rate (*A*) (mean ± standard error) in rice varieties infested by *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments within each post-infestation time with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

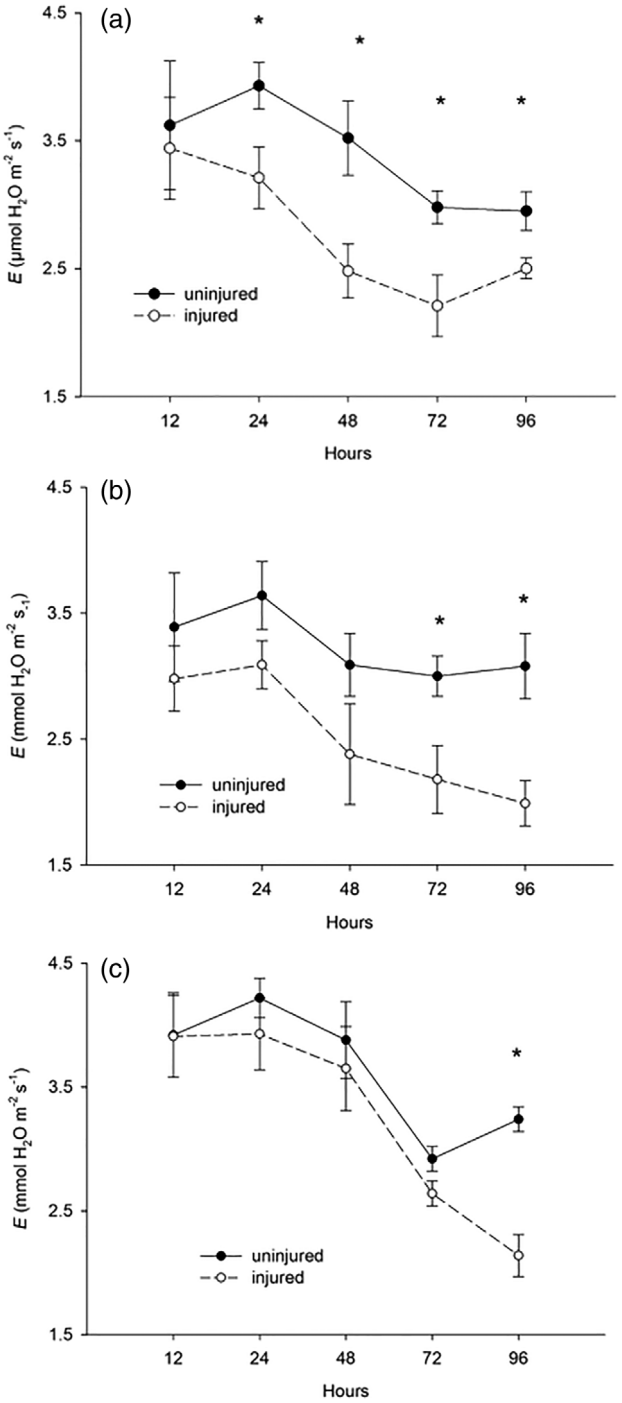

Figure 2. Transpiration rate (*E*) (mean ± standard error) in rice varieties infested with *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments within each post-infestation time with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

Considering chlorophyll content, the Xingu and Canela de Ferro genotype plants infested with rice stalk stink bugs showed a significantly lower SPAD index (Fig. 5). In the Xingu genotype, the average reduction in chlorophyll content 72 h after infestation was 20% ($t_{1,30} = 3.94$, $P = 0.003$). In the Canela de Ferro genotype, the reduction was 25% 48 h after infestation ($t_{1,30} = 4.76$, P 0.001). In contrast, injured Primavera genotype plants presented no significant reduction in chlorophyll content compared to uninjured plants.

The enzymes of the cellular antioxidant defense system, SOD, CAT and POX, were found to be more active when rice plants were infested with rice stalk stink bug (Figs 6, 7 and 8). Concerning SOD,

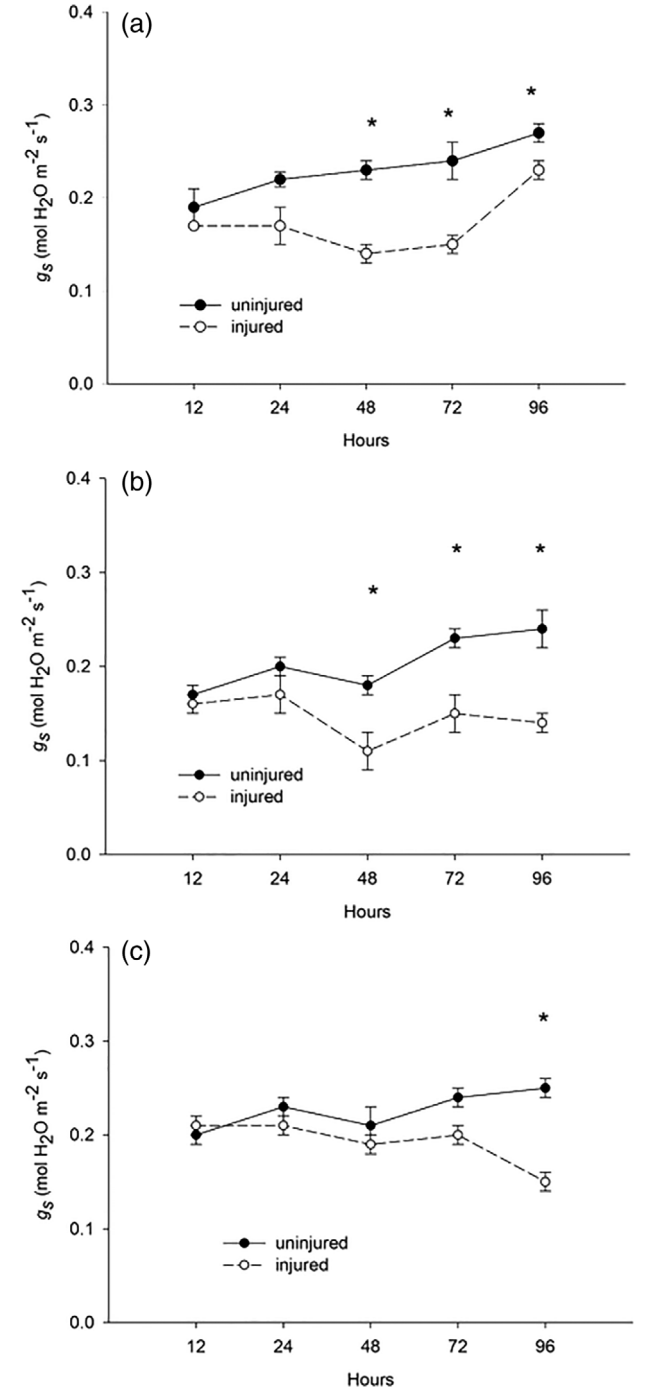

Figure 3. Stomatal conductance (g_s) (mean ± standard error) in leaves of rice varieties infested by *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments within each post-infestation time with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

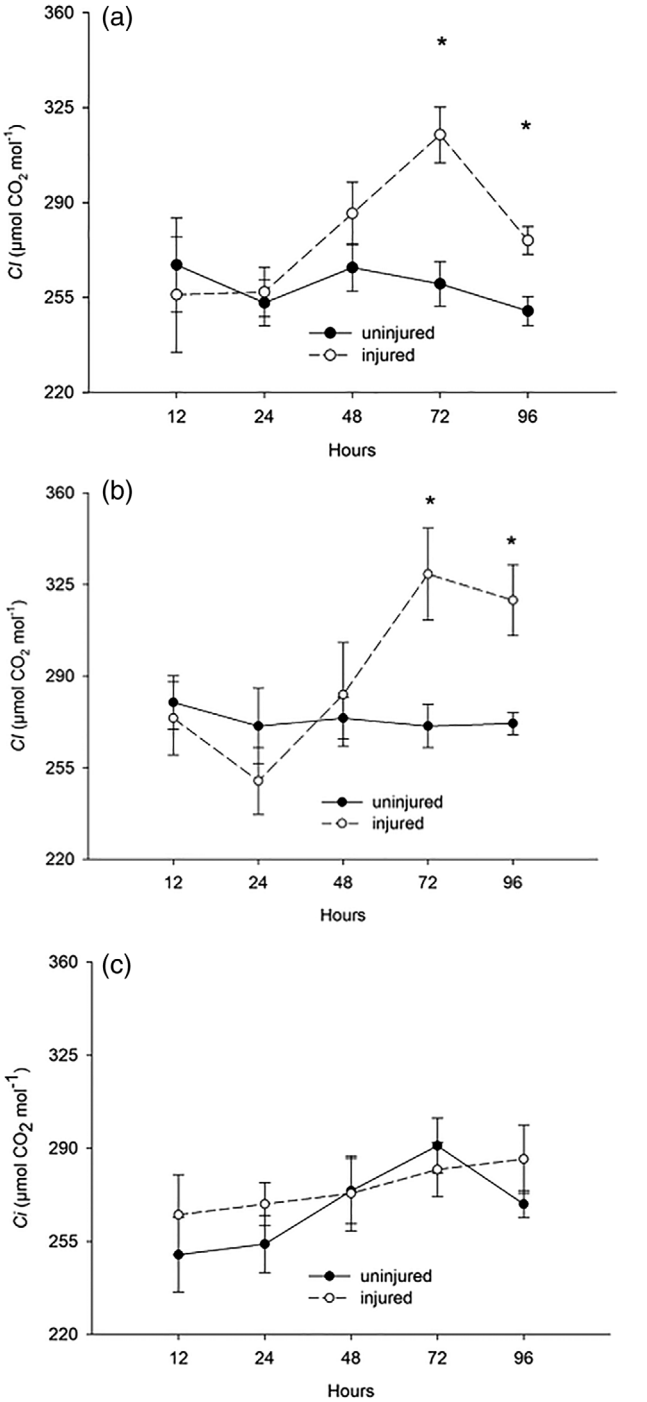

Figure 4. Internal concentration of carbon (CI) (mean ± standard error) in rice varieties infected by *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments within each post-infestation time with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

injured plants of Xingu, Canela de Ferro and Primavera genotypes showed significantly higher specific activity (60%) at 96 ($t_{1,30} = 2.95$, P 0.006), (30%) 48 ($t_{1,30} = 2.06$, $P = 0.0498$) and (61%) 12 h ($t_{1,30} = 4.11$, $P < 0.001$) after infestation, respectively. For CAT, injured plants of the Xingu, Canela de Ferro and Primavera genotypes showed significantly higher specific activity (48%) 48 ($t_{1,30} = 1.87$, $P = 0.008$), (69%) 24 ($t_{1,30} = 3.11$, $p = 0.0075$) and (45%) 48 h ($t_{1,30} = 2.89$, $P = 0.0117$) after infestation, respectively. For POX, injured plants of the Xingu, Canela de Ferro and Primavera genotypes showed significantly higher specific activity (56%) 48 ($t_{1,30} = 2.76$, $P = 0.0116$), (22%) 72 ($t_{1,30} = 2.97$, $P = 0.005$) and (28%) 24 h ($t_{1,30} = 2.56$, $P = 0.0154$) after infestation, respectively.

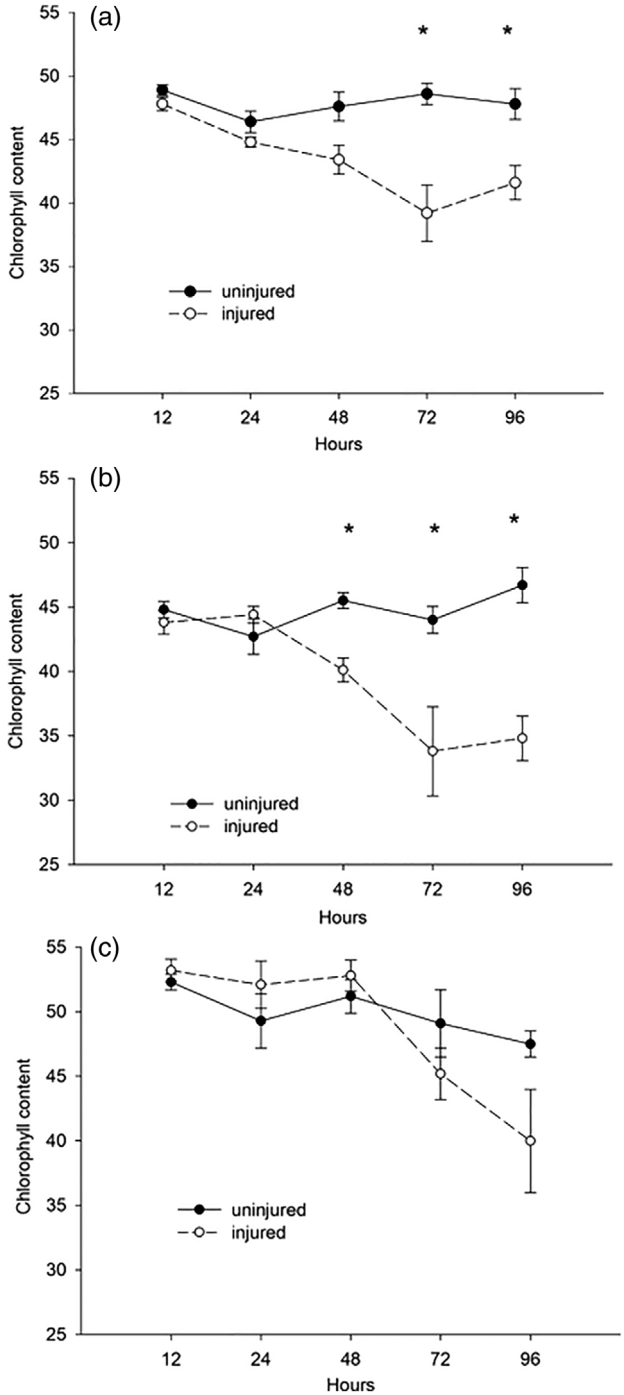

Figure 5. Chlorophyll content (SPAD) (mean ± standard error) in leaves of rice varieties infested with *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments within each post-infestation time with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

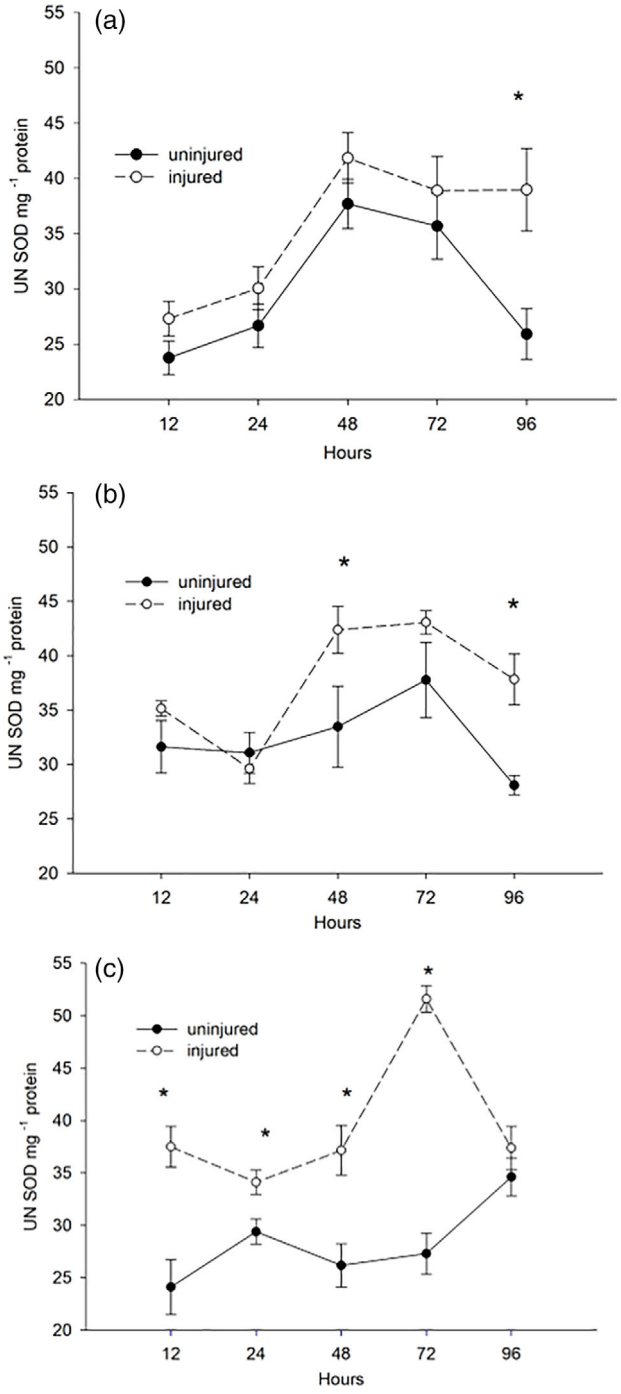

Figure 6. Superoxide dismutase (SOD) (UN SOD mg^{-1} protein) (mean ± standard error) enzyme activity in the sap of rice varieties infested with *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments within each post-infestation time with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

4 DISCUSSION

In our study, we characterized the tolerance of rice to rice stalk stink bugs, measuring the agronomic performance of plants attacked by rice stalk stink bugs and the physiological responses of the injured plants.

The agronomic performance of Xingu, Canela de Ferro and Primavera cultivated in the presence and absence of rice stalk stink bugs had different responses to pest damage. The Primavera genotype performed differently from the Xingu and Canela de Ferro genotypes, presenting no significant reduction in GY per plot or the NFG, in addition to no significant increase in percent SS in injured plants compared with uninjured plants. Previous studies have shown that tolerant insect-injured plants have increased growth and nitrogen physiology,[49] increased plant

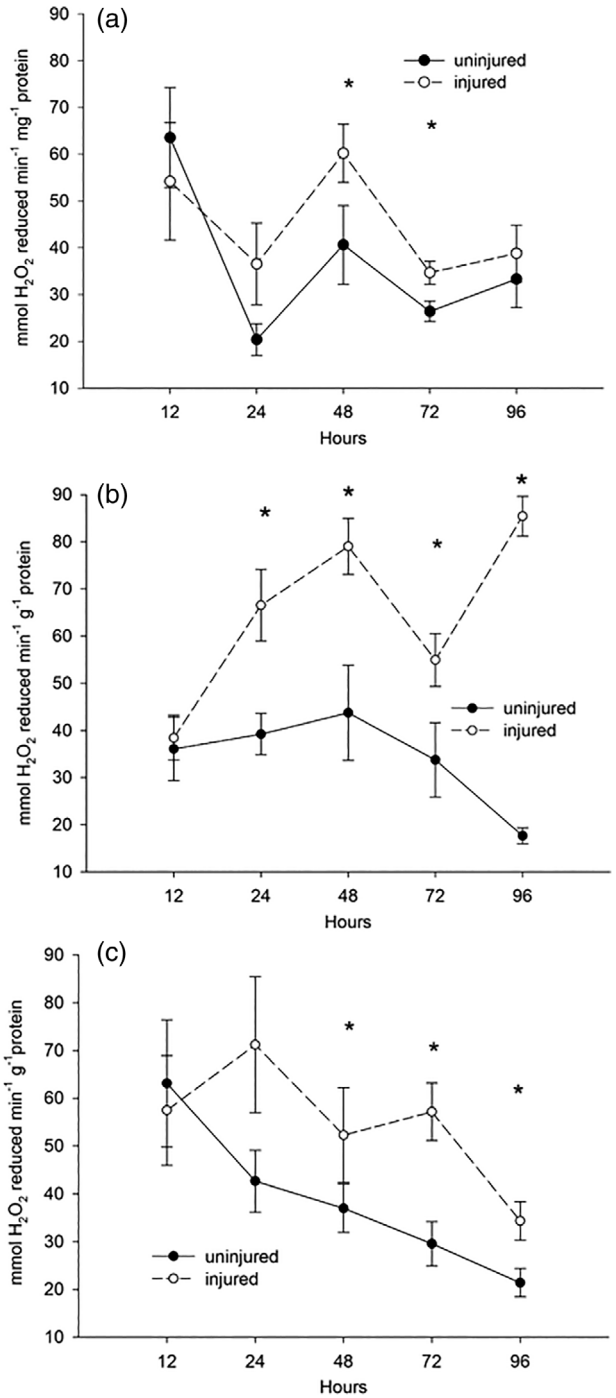

Figure 7. Catalase (CAT) activity (mmol H_2O reduced $min^{-1}g^{-1}$protein) (mean ± standard error) in the sap of rice varieties infested with *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments within each post-infestation time with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

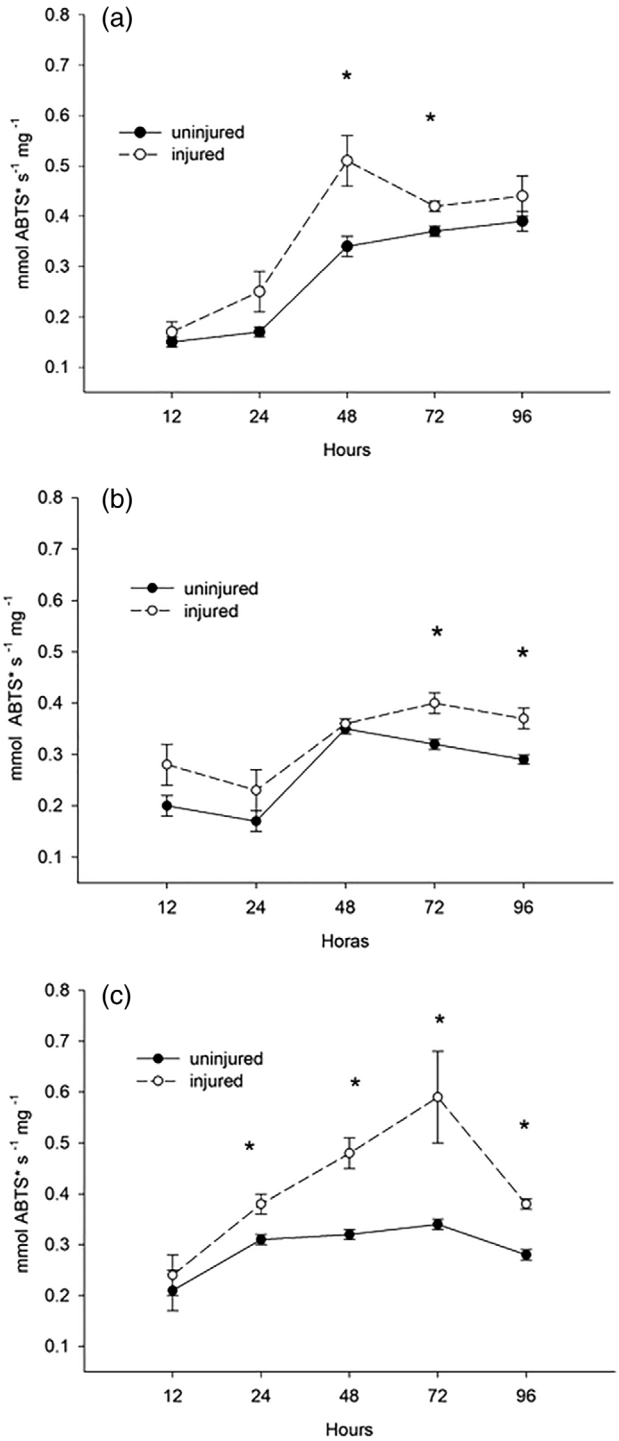

Figure 8. Peroxidase (POX) activity (mmol ABTS* s^{-1} mg^{-1}) (mean ± standard error) in the sap of rice varieties infested with *Tibraca limbativentris*. (a) Xingu, (b) Canela de Ferro and (c) Primavera. Treatments with an asterisk (*) were statistically distinguished by *t*-tests ($P < 0.05$) within each post-infestation time.

vigor[50] or increased photosynthetic activity in their undamaged parts to compensate for the damage caused by herbivory.[51,52]

In parallel, the leaf gas exchange performance of the Primavera genotype was minimally affected by the damage caused by the rice stalk stink bug compared to the response of injured plants of the Xingu and Canela de Ferro genotypes. The carbon assimilation values for Primavera leaves (12 to 72 h after infestation) did not differ, with a mean relative photosynthesis value of 91% compared to the uninjured plants. As the infestation increased, a moderate decrease (41%) in relative photosynthesis was observed 96 h after infestation. Xingu and Canela de Ferro showed an abrupt reduction (56% and 65%) 24 and 48 h after infestation. Changes in vascular tissue may explain the reduction

in the photosynthetic rate in plants infested with the rice stalk stink bug, as this insect lodges at the base of the stalk of rice plants and sucks the sap by inserting stylets between the conductive vessel cells.[9,53] Nonetheless, we cannot ignore the possibility that the chemical compounds in insect saliva affect photosynthetic tissues.[53,54] Photosystem II physiological changes can occur after the destruction or inactivation of the pigment responsible for light capture, and without the inductive amplification of the captured light, there are no free electrons to conduct the biochemical step of photosynthesis.[55,56]

With effective plant–insect interactions, the vascular tissue is compromised, affecting not only sap translocation but also xylem current, which can be observed, in this study, through transpiration and stomatal conductance reduction. The first effect of stomatal closure is a decrease in mesophyll CO_2 and in net photosynthesis, in part due to a decrease in the synthesis of rubisco and in its carboxylation activity and efficiency, or both.[57] However, stomatal conductance is not controlled by any one factor alone but by a complex interaction of several leaf internal and external factors.[58] As stomatal conductance has a close relationship with leaf transpiration, since water exits the leaf through the stomata, an abrupt reduction in both was observed in Xingu and Canela de Ferro during the first hours of infestation. Furthermore, the stomata usually maintain a constant internal partial CO_2 pressure relative to the external pressure. CO_2 concentration is defined as the balance between CO_2 consumption (photosynthesis) and replacement (external flux, respiration, and photorespiration).[59,60] Thus, infested Xingu and Canela de Ferro plants showed increased concentrations of internal CO_2 in their leaves, which may be associated with increased respiratory and photorespiratory rates.[53,61] The plant breathes more due to the need for extra energy production to repair the structures damaged by the insect.[9]

However, increased production of secondary colms, preexisting high levels of carbon storage in roots, and the ability to reallocate carbon after injury from roots to shoots,[60] may have had a significant impact on the stability of the grain yield of Primavera, since these are additional types of compensatory growth mechanisms that allow plants to recover from herbivore attack, although these components have not been evaluated in our study. According to Lemoine *et al*.[62] senescence and reserve mobilization are integral components of plant development and basic strategies used in stress mitigation.

Leaves normally have a number of chloroplasts and photosynthetic pigments (chlorophyll a, chlorophyll b, carotene, and xanthophyll), far beyond what is necessary,[62] so they become more opaque when infested by pests. Our results indicated that stink bug attack reduces chlorophyll content at levels detectable by SPAD and that this reduction depends on the degree of tolerance of the genotype in question. Infested plants of Xingu and Canela de Ferro presented a reduced chlorophyll content, while the Primavera genotype showed no difference. Altered SPAD levels due to attacks by piercing-sucking insects were also detected by Goławska *et al*.[63] and Jesus *et al*.,[10] who studied four leguminous species attacked by aphids.

The attack of insects on plants causes an oxidative explosion characterized by the production of ROS,[64] which, if produced in large quantities, causes deleterious effects on cells.[25] According to Lei and Zhu-Salzman,[65] rapid H_2O_2 production upon aphid infestation in the bik1 mutant suggests that the BIK1 (BOTRYTIS-INDUCED KINASE1) gene, encoding a receptor-like cytoplasmic kinase in Arabidopsis, affects ROS homeostasis. RESPIRATORY BURST OXIDASE HOMOLOGUE (AtRBOH) genes in Arabidopsis encode NADPH oxidases involved in ROS production in response to infection by bacterial and fungal pathogens, particularly AtRBOHD and AtRBOHF. Arabidopsis serine/threonine kinase OXIDATIVE SIGNAL-INDUCIBLE1 (AtOXI1) and zinc finger protein AtZat12 are both marker genes for ROS signaling. Arabidopsis CATALASE1 (AtCAT1) and CATALASE2 (AtCAT2) can detoxify H_2O_2 and are induced by abiotic stresses. Cytosolic ASCORBATE PEROXIDASE1 (AtAPX1) can also scavenge H_2O_2.

As a form of defense, plants have an apparatus of antioxidant system enzymes that minimize the effect of ROS.[23,66] Several studies have described the ability of tolerant genotypes to increase the activity of antioxidant system enzymes when challenged by insect damage.[10,22,24,67,68] In this study, Primavera plants had increased specific SOD activity from the first hours of insect-plant contact, differing significantly from Xingu and Canela de Ferro plants, which showed an increase in SOD activity 96 and 48 h after infestation, respectively. SOD is the enzyme that is the first line of defense against the toxic effects of ROS, as it is responsible for the displacement of the superoxide radical in H_2O_2 and molecular oxygen.[69] Sytykiewicz *et al*.[70] described a significant increase in superoxide anion radicals ($O_2^{-\cdot}$) in maize seedlings infested with *Rhopalosiphum padi* (bird cherry-oat aphid).

In the cascade of detoxification reactions, the enzymes CAT and POX act next, as they are responsible for H_2O_2 demutation in water and oxygen.[21,71] Primavera genotype plants showed high activity at most evaluation times, showing that their attack perception and detoxification mechanisms were immediately triggered following the attack. However, the activity levels of these enzymes in insects are believed to be crucial factors in determining their resistance to a broad spectrum of toxic chemicals.[65]

Thus, it is plausible that integration of these processes might be involved in attenuation of the defense response, maintaining higher levels of ROS mitigating systems, compensation of photosynthates lost due to insect herbivory and renewed growth of the meristems and maintained grain yield in the Primavera genotype. Screening studies of the gene bank to identify rice genotypes tolerant to the rice stalk stink bug, as well as molecular biological screening to identify and characterize tolerance genes in rice plants, should be conducted to support the rice breeding program to develop resistant cultivars. In addition, gas exchange and chlorophyll content are physiological attributes that can be used to identify rice genotypes more tolerant to rice stalk stink bugs, as they are sensitive, non-destructive methods for detecting pest damage and are easy to use in field conditions.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: ACSA, FGJ, TH-M, ACL and JAFB, Performed the experiments: ACSA, ACL and JAFB. Analyzed the data: ACSA. Wrote the paper: ACSA, FGJ, TH-M, ACL and JAFB. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in this research.

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