Agr. Nat. Resour. 57 (2023) 635–646

AGRICULTURE AND
NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Efficacy of silicate elicitors and their docking studies on managing bacterial leaf blight in glutinous rice plants

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Article Info

Article history:
Received 12 April 2023
Revised 14 July 2023
Accepted 25 July 2023
Available online 31 August 2023

Keywords:
Disease reduction,
Docking studies,
Enzyme,
Induced resistance,
Xanthomonas oryzae

Abstract

Importance of the work: Bacterial leaf blight (BLB) is a serious disease in glutinous rice plants. Silicate elicitors could help to manage and reduce problems caused by BLB.
Objectives: To evaluate the effect of Si in different forms ($CaSiO_3$, K_2SiO_3 and Na_2SiO_3) and to understand resistance mechanisms against BLB.
Materials & Methods: The expression of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) was assessed. *In silico* research on interactions between Si and PAL/PPO was conducted and the endogenous salicylic acid accumulation and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) density inside glutinous rice plants were assessed. The efficacy of silicate elicitors against BLB was evaluated in field conditions.
Results: The Si elicitors helped glutinous rice plants resist BLB in field conditions. They improved disease resistance mechanisms, including salicylic acid accumulation, and activated defense enzymes, such as PAL and PPO. The amino acids LYS45, LEU56, ARG66 could be active sites of PAL, while the amino acids ASP86, PRO89, ARG103 could be active sites of PPO in forming a strong hydrogen bond with Si. Such interactions lead to the formation of active components of PAL or PPO, contributing to disease resistance. Furthermore, applying Si increased the density of *Xoo*, maintained the amylose percentage and increased the yield.
Main finding: Among the Si elicitors, K_2SiO_3 and $CaSiO_3$ showed higher efficacy than Na_2SiO_3 in glutinous rice plants. Based on the known studied literature, this was the first report showing the beneficial efficacy of silicate elicitors in glutinous rice plants.

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https://doi.org/10.34044/j.anres.2023.57.4.08

Introduction

The Mekong Delta is one of Vietnam's main rice production areas (Maitah et al., 2020). Intensification of rice cultivation and climate change has recently led to outbreaks of many insect pests and plant diseases (Heeb et al., 2019; Shivappa et al., 2021). Bacterial leaf blight (BLB) caused by its agent *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases of rice plants (Chen et al., 2019), as well as glutinous rice plants (Wongkhamchan et al., 2018). In the north of Vietnam, during the phenotypic screening of the local glutinous rice accessions in the 2016 autumn season, Tung et al. (2018) reported that 19 out of 56 accessions contained resistance genes, including *Xa4*, *xa5* and *Xa7*. In addition, glutinous rice cv. CK92 (also known as cv. AG) is susceptible to BLB (Anh et al., 2017). However, there has been no published research on BLB resistance in the Mekong Delta in southern Vietnam.

Glutinous (or "sticky") rice (*Oryza sativa* var. *glutinosa*) is a source of valuable, gluten-free carbohydrates, containing over 90% amylopectin and less than 10% amylose. There are 6,484 glutinous rice varieties in Asia, with Thailand, Lao PDR and Vietnam being the primary production areas of glutinous rice globally (Sattaka, 2016; Sengsourivong and Ichihashi, 2019). In Vietnam, glutinous rice constitutes approximately 10% of total rice production and consumption (Trudel, 2012). It is used in many social and family activities, including weddings, rituals, folk festivals, ancestor worship and funerals (Nguyen, 2001). Glutinous rice farmers are mostly small-scale growers, with an average cultivation area of 0.15 ha and an average yield of 3.2 t/ha (Sattaka et al., 2017). An Giang province is the most extensive glutinous rice-growing area in the Mekong delta, followed by Ca Mau province (Sattaka et al., 2017).

Many methods exist to control disease damage and outbreak, such as disease resistance cultivars and physical and chemical control. The use of resistant cultivars is one of the safest methods; however, resistance to pathogens can quickly break down due to the characteristically rapid rise of mutations among bacterial populations and the emergence of new, resistant, bacterial strains (Leach et al., 2001). The broad spectrum BLB-resistance gene is *Xa21* gene (Sagun et al., 2019). Using chemical bactericides to control BLB is quick and efficient; however, this raises production costs, pollutes the environment and causes harm to human health and agroecology. In recent years, induction of plant resistance against diseases (induced resistance) has been an advanced approach to controlling BLB. Induced resistance is a sustainable and ecological approach to control plant diseases, in which elicitors, both biotic and abiotic, are used to stimulate the crop's defense system to reduce the negative effect of phytopathogen infection (Walters et al., 2013).

Plants can receive diversified environmental signals that prime them to react to their surroundings (Oanh and Korpraditskul, 2006). Stimuli may be abiotic or biotic, including pathogen-derived stimuli, plant growth-promoting rhizobacteria/fungi, mycorrhizae, probenazole, salicylic acid (SA), and silicate compounds (Mauch-Mani et al., 2017; Koodkaew et al., 2020; Hata et al., 2021). Later, when being attacked, the recognition of pathogenic microorganisms by host plants leads to multiple outcomes, including activation of rapid ion fluxes, the action of kinase cascades, generation of reactive oxygen species, the production of endogenous signals, such as SA, jasmonic acid and ethylene, accumulation of polyphenols,; induction of a hypersensitive response, expression of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) and the production of defense proteins and phytoalexins (Mishra et al., 2012; Le Thanh et al., 2017; Yin et al., 2021). Defense enzyme induction is also crucial in the underlying disease resistance process. Singh et al. (2021) showed that ascorbic acid (20 mM), dehydroascorbic acid (20 mM) and ascorbate oxidase (20 mM) could help rice plants (*Oryza sativa* subsp. *japonica*) to increase the concentration of PAL against the parasitic root-knot nematode. Stanley-Raja et al. (2021) reported that fungal chitosan helped rice plants to produce direct toxicity to *Xoo*, with BLB severity being reduced by 39.9% and in addition, the treatment of fungal chitosan increased the levels of PAL by 67.3% compared with the untreated control. Zhang et al. (2012) reported that Si application increased the concentration of phenolics and the enzyme levels of PPO and PAL in the sensitive rice cultivar Ningjing 1. SA is the primary signal of systemic resistance during induced resistance by abiotic stimuli (Walter et al., 2007).

Silicon (Si), a macroelement, plays a crucial role in plant life by improving plant growth and yield, especially the underlying biotic and abiotic stress conditions. For example, it increased the induced resistance of sugarcane plants, limiting damage from *Scirpophaga excerptalis* Walker (sugarcane top borer) (Rahardjo et al., 2021). In *Arabidopsis*, Si stimulated disease resistance to powdery mildew caused by the isolate *Golovinomyces cichoracearum* UCSC1 (Wang et al., 2020). In Kalanchoe 'Peperu', different silicon sources such as $CaSiO_3$, K_2SiO_3, and Na_2SiO_3, were assessed at concentrations of 50 mg/L. The results showed that $CaSiO_3$ increased the shoot tissue and chlorophyll contents compared to the Na_2SiO_3 or K_2SiO_3 treatments (Son et al., 2012). Applying Na_2SiO_3 at 2 mM in cucumber increased its seedling resistance to wilt disease caused by *Fusarium oxysporum* f.sp. *cucumerinum* Owen (Zhou et al., 2018). In rapeseed, K_2SiO_3 at 10 and 100 mM induced defense responses against sclerotinia stem rot (*Sclerotinia sclerotiorum*) with reductions in the lesion size of 86% and 38.3%, respectively (Feng et al., 2021). In bananas, an amendment with K_2SiO_3 and *Bacillus subtilis* induced a higher total chlorophyll content, an increased rate of leaf gas exchange and biochemical changes for controlling *Fusarium* wilt disease infection (Zakaria et al., 2023). In rice, Si at 1.5 mM improved resistance to sheath blight disease (*Rhizoctonia solani*) in the sensitive rice cultivar Ningjing 1, with a reduction of 2.96 units (Zhang et al., 2012). Finally, root-applied Si enhanced rice resistance to nematode root-knot disease (*Meloidogyne graminicola*) (Zhan et al., 2018). Nonetheless, there has been no known published research on Si application against bacterial diseases *(Xoo)* in glutinous rice plants.

Therefore, the present research evaluated the effects of foliage-sprayed silicate compounds in different forms ($CaSiO_3$, K_2SiO_3 and Na_2SiO_3) in glutinous rice plants to investigate whether it induced resistance against *Xoo* and the defense mechanisms underpinning any induced resistance of glutinous rice plants.

Materials and Methods

Source of virulence pathogen, glutinous rice and elicitors

The aggressive *Xoo* strain ST6 was provided by the Laboratory of Biological Control, Department of Plant Protection, Can Tho University, Vietnam and used for all experiments in net-house conditions. The stock of *Xoo* was cultured on King's B medium (10 g peptone, 1.5 g K_2HPO_4, 1.5 g $MgSO_4.7H_20$, 10 mL glycerol, 1 L distilled water, without agar) for 48 hr, then diluted with sterilized deionized water to adjust its density to 1 × 10^8 colony forming units(CFU)/mL. The optical density of the bacterial suspension was measured using a Spectro SC Spectrophotometer (Helios Alpha; Australia) at a wavelength of 600 nm.

Seeds of glutinous rice cv. CK92 were provided from The Agricultural Extension Station Phu Tan, An Giang province, Vietnam and were used for all experiments in this research. This is the most widely used glutinous cultivar in An Giang province, with a growing time of approximately 105 d.

The elicitors used in the research were calcium silicate ($CaSiO_3$; Merck 10101-39-0), potassium silicate (K_2SiO_3; Merck, 1312-76-1) and sodium silicate (Na_2SiO_3; Merck, 10213-79-3).

Evaluation of elicitor effect on expression of phenylalanine ammonia-lyase and polyphenol oxidase in glutinous rice plants

The experiment was conducted in a completely randomized design (CRD), with four treatments (Table 1), four replications and two plants per replication. The soil was chopped, sun-dried for 15 d and then transferred into plastic pots (4 kg of soil per pot). The glutinous rice seeds were germinated and grown in plastic pots. Fertilizer was applied following the usual dosage for glutinous rice cultivation for 1 ha ($145N\text{-}46P_2O_5\text{-}60K_20$), corresponding to $0.0019N\text{-}0.0006P_2O_5\text{-}0.0008K_20$ per 40 cm diameter pot. The elicitors or water were sprayed on the foliage on days 25 and 45 after growing (DAG). At 50 DAG, 10 fully expanded leaves of each rice plant were chosen and marked. Leaves were tip-cut and dipped into the prepared suspension of *Xoo*. The plants were incubated in an inoculation room in dark conditions at 25^{o}C, and a relative humidity of 95 ± 3%, for 48 hr. After that, the pots were placed in the net house.

Leaves were collected at 0 hr, 3 hr, 6 hr, 9 hr and 12 hr after inoculation (HAI), immediately dipped into liquid nitrogen and stored at -80 °C. Then, 0.1 g of leaf sample was ground to a fine powder in a chilled mortar with 3–5 mL of liquid nitrogen. The powder was placed in an Eppendorf cylinder containing 1.5 mL borate buffer 0.1 M, pH 8.7 (for the extraction of PAL) and buffer Na-P 0.1 M, pH 6.5 (for the extraction of PPO), and gently mixed. The mixture was centrifuged at 10,000 revolutions per minute (rpm) for 30 min at 4°C. The upper liquid was removed using a pipette and used to assess the expression of PAL or PPO (Agarwal et al., 2012; Nisha et al., 2012).

Assessment of expression of phenylalanine ammonia-lyase

First, a blank sample containing 0.7 mL borate buffer 0.1 M (pH 8.7), 1 mL L-Phe 1.0 M and 0.15 mL water was measured at an optical density (OD) of 290 nm and tared back to zero. The treatment sample had 0.7 mL borate buffer 0.1 M (pH 8.7), 1 mL L-Phe 0.1 M and 0.15 mL crude enzyme liquid. The reaction was performed at 37°C for 40 min and then stopped by adding 0.2 mL HCl 5.0 N (Sadasivam and Manickam, 1996; Wardatun et al., 2017). The quantity of the enzyme PAL was determined on the standard OD curve versus the concentration of trans-cinnamic acid (PAL product). The PAL quantity was presented in units of micrograms per protein per minute (Sirin et al., 2016; Wardatun et al., 2017). The experiment was conducted in triplicate.

Assessment of expression of polyphenol oxidase

The blank sample consisted of 1.75 mL catechol 0.2 M and 0.15 mL Na-P buffer 0.1 M (pH 6.5). It was measured at OD 490 nm and tared back to zero. The treatment sample had 1.75 mL catechol 0.2 M and 0.15 mL crude enzyme liquid. The reaction was recorded every 30 s, until 2 min (Agarwal et al., 2012; Nisha et al., 2012). The quantity of enzyme PPO was determined on the standard OD curve versus the concentration of gallic acid (PPO product). The PPO quantity was also presented in units of micrograms per protein per minute (Agarwal et al., 2012). The experiment was conducted in triplicate.

In silico research on interaction between Si and phenylalanine ammonia-lyase or polyphenol oxidase

The sequences of PAL and PPO from *Oryza sativa* (Uniprot_KB Accession Ids: A2X7F7 and A5X3J7) were collected and their structure was predicted using homology modeling methods from the protein databank. For domain selection, sequences of PAL and PPO were submitted to the SBASE server (pongor.itk.ppke.hu). Then, the collected sequences were submitted to the BLAST server, using the PDB database for template selection. The template sequences were compared and models were generated using the Modeller 9v7 software (Eswar et al., 2007). Next, the protein was stabilized by adding hydrogens to the 3D structure through molecular dynamics (MD) simulation studies. The MD simulations of the predicted model were performed using the CHARMM27 and NAMD2.8 force fields (Guo et al., 2022). The algorithm of multiple-time-stepping was computed, including short-range forces and long-range electrostatics at each level. The MD procedure was performed at the new positions to receive new velocities using Hamilton's equations of motion and a final model containing the advanced thermodynamic properties information was achieved (Thepbandit et al., 2021). The structures of PAL and PPO with lesser root mean square deviation value were obtained in the MD studies. Then, the structures of PAL and PPO were predicted based on the Ramachandran plot using the PROCHECK server to test the stereochemical quality of the protein structures. Finally, the ERRAT server was used to evaluate the environment to reveal the active sites and to dock to the SA (Kumar et al., 2017).

Active site identification phenylalanine ammonia-lyase and polyphenol oxidase

The possible PAL and PPO binding sites were checked based on template structural comparisons or model building using the CASTp server (sts.bioe.uic.edu/castp; Papathoti et al., 2020).

Docking studies with silicon oxide

The advanced binding conformation of Si was determined using the GOLD 3.0.1 software following docking studies (Daddam et al., 2020a). The Si ion was docked to the active sites of PAL and PPO to evaluate the possibility of enhancing the activity of Si. Then, individual binding poses of each protein-ion complex were selected, from which the binding energies of individual binding poses were studied. The most energetic conformation of the protein-ion complex was selected to analyze docking traits (Daddam et al., 2020b).

Analysis of endogenous salicylic acid accumulation

The experiment was carried out using a CRD, with four treatments (Table 1), four replications and two plants per replication, repeated in triplicate. Preparation of the soil and glutinous rice seeds, application of fertilizer and suspension of *Xoo* were conducted as described above. At 7 d after inoculation (DAI), leaf samples were collected and immediately dipped into liquid nitrogen and then stored at -80°C.

From each sample, 0.5 g was homogenized in 1 mL of extraction buffer containing an equal volume ratio of acetic acid glacial-to-methanol-to-deionized water. The sample was centrifuged at 10,000 rpm, at 4°C for 10 min. Then, 0.5 mL of supernatant was mixed with 0.02 M $NH_4Fe(SO_4)_2$ and incubated for 5 min at 30 ± 1°C. The endogenous SA was calculated based on the SA standard and absorbance of the mixture at 530 nm (Chithrashree et al., 2011).

Table 1 Treatment for experimental assessment of enzyme

Treatment	Concentration	Inoculation of *Xoo*
$CaSiO_3$	Spray $CaSiO_3$ 0.01% at 25, 45 DAG	Inoculation
K_2SiO_3	Spray K_2SiO_3 0.01% at 25, 45 DAG	Inoculation
Na_2SiO_3	Spray Na_2SiO_3 0.01% at 25, 45 DAG	Inoculation
Water control	Spray water at 25, 45 DAG	Inoculation

DAG = days after growing

Evaluation of effect of elicitor inhibition of Xoo density inside glutinous rice plants

The experiment was conducted in a CRD, with four treatments (Table 1), five replications and two plants per replication, repeated in triplicate. Two leaves per plant were sampled at 0 DAI, 7 DAI, 10 DAI, 20 DAI and 30 DAI (corresponding to 50 DAG, 57 DAG, 60 DAG, 70 DAG and 80 DAG). Each sample was surface-sterilized using 95% ethanol (volume per volume) and then ground and diluted in sterile 10 mM $MgCl_2$. The serial dilutions were spread on nutrient glucose agar Petri dishes. The Petri dishes were incubated at 27±2°C for 24 hr to count the appearance of *Xoo* colonies (using a minor modification from Le Thanh et al., 2017).

Evaluating effect of elicitors against bacterial leaf blight in field conditions

The experiment was performed in a randomized complete block design, with five treatments ($CaSiO_3$, K_2SiO_3, Na_2SiO_3, a positive control (Silimax) and a water control) and four replications of a 24 m^2 per plot (3 m × 8 m). The glutinous rice field was in Phu Tan district, An Giang province, Vietnam. The elicitors were sprayed onto the foliage at 25 DAG and 45 DAG. On the positive control treatment, the chemical bactericide was applied following the manufacturer's instructions. Fertilizer was applied following the usual dosage for glutinous rice cultivation for 1 ha ($145N\text{-}46P_2O_5\text{-}60K_20$). Selective insecticides were applied when insects caused considerable damage. There was no application of pesticides for pathogens. Rice plants were infected by the natural infection of *Xoo*. BLB disease scores were recorded at 67 DAG, 74 DAG, 81 DAG and 88 DAG, following the IRRI-SES scale (International Rice Research Institute, 1996). In each field plot, five positions were chosen where the BLB scores were recorded on all leaves of 10 plants per position. A score of 1 indicated BLB disease covering 1–5% of leaf area, while scores of 3, 5, 7 and 9 indicated coverage of 6–12%, 13–25%, 26–50% and more than 51% of the leaf area, respectively. Disease severity (DS) was calculated using Equation 1:

$$DS(\%) = \left(\frac{\sum_{i=1}^{n} r_i}{n \times m} \right) \times 100\% \tag{1}$$

where *n* is the total number of leaves graded per plant, *r* is the set of numerical ratings and *m* is the maximum grade on the disease scale.

The rice seeds were collected from 5 m^2/plot and cleaned and measured for seed humidity (H_0) and scaled seed weight (W_0). The yield was calculated using Equation 2 (Ministry of Agriculture and Rural Development, 2011):

$$W_{14\%} = [W_0 \times (100 - H_0)]/86 \tag{2}$$

The amylose percentage was determined using the protocol of Cagampang and Rodriguez (1980). Briefly, 50 mg of fine glutinous rice powder (ground from collected rice seeds) was transferred to a 50 mL Falcon tube and then added with 0.5 mL ethanol 95% and gently shaken. After that, 9.5 mL NaOH 1N was added. The solution was boiled for 10 min, gently shaken and left to stand overnight at room temperature. In the next step, 100 μL of solution was pipetted into a 20 mL volumetric flask. In the blank flask, 100 μL NaOH 1N was added instead of the 100 μl solution. Deionized water was added to fill one-half of the flask and then gently shaken. Later, 250 μL of iodine was added, gently shaken and then deionized water was added until the solution reached the fill line of the volumetric flask. The solution was poured into a new Falcon tube, shaken and the OD was measured at a wavelength of 580 nm. The standard curve of amylose was prepared following the procedure for the National Standard of Rice—Determination of Amylose Content (Ministry of Agriculture and Rural Development, 2017).

Statistical analysis

The data from all experiments were analyzed using analysis of variance and means were compared based on Duncan's multiple range test (DMRT) at $p < 0.05$ by using the SPSS 20.0 software (IBM; USA). For the net-house conditions, the experiments were repeated in triplicate and in each experiment, treatments had 4–5 replications. The three datasets (three times) of each experiment were performed separately. For each experiment, homogeneity and normality tests were carried out. The results of one-repeated experiment were chosen for presentation in the current report. For the field conditions, the experiment was repeated once, with five treatments and four replications per treatment.

Results

Activities of phenylalanine ammonia-lyase and polyphenol oxidase

The activity levels of the PAL and PPO enzymes in glutinous rice plants after treatment with elicitors were characterized at 0 HAI, 3 HAI, 6 HAI, 9 HAI and 12 HAI.

The results of PAL activity are shown in Table 2. At 0 HAI, the PAL concentrations of these elicitor treatments gained approximately 1.30–2.95 µg/protein/min, which were significantly higher than the water control (0.25 µg/protein/min). At 3 HAI, two treatments ($CaSiO_3$ and K_2SiO_3) had PAL activity levels (2.64 and 7.73 µg/protein/min, respectively) that were significantly higher than the water control (0.70 µg/protein/min). In contrast, the Na_2SiO_3 treatment was not significantly different from the control. At 6 HAI, 9 HAI and 12 HAI, the PAL levels increased and were still significantly higher than those of the water control. At 12 HAI, the PAL concentrations of $CaSiO_3$, Na_2SiO_3, K_2SiO_3 and the water control were 20.74 µg/protein/min, 10.42 µg/protein/min, 32.55 µg/protein/min and 2.20 µg/protein/min, respectively (Table 2).

The PPO values of the K_2SiO_3 treatment were significantly higher than those of the water control at all measurement times, while those of the other two elicitors were not. The PPO concentrations of K_2SiO_3 gained 1,347.92–4,297.92 µg/protein/min, while the levels of the water control were 258.33–997.92 µg/protein/min during 0–12 HAI. The next-most effective treatment was $CaSiO_3$, which had PPO concentrations significantly higher than the water control at 9 HAI and 12 HAI (gains of 1,585.42 and 2,164.58 µg/protein/min, respectively). The last elicitor, Na_2SiO_3, had PPO concentrations of approximately 316.92 –1,687.50 µg/protein/min during 0–12 HAI, which were not significantly different from the water control (Table 3).

Modeling of phenylalanine ammonia-lyase

The PAL and PPO sequences from *Oryza sativa* shared 86% and 88.5% similarity with the respective proteins from *Arabidopsis*. These two sequences showed well-conserved regions in alignment. The modeled structure showed three helices in the quaternary form. The MD simulation studies, Ramachandran plot server and PROCHECK confirmed the reliability of the structure.

Table 2 Expression of enzyme phenylalanine ammonia-lyase (PAL) in glutinous rice plants after treating elicitors

Treatment	PAL activity at hours after inoculation (µg/protein/min)				
	0 HAI	3 HAI	6 HAI	9 HAI	12 HAI
$CaSiO_3$	1.30 ± 0.01^{a}	2.64 ± 0.02^{b}	5.19 ± 0.02^{ab}	8.33 ± 0.05^{b}	20.74 ± 0.03^{ab}
Na_2SiO_3	1.45 ± 0.02^{a}	2.49 ± 0.03^{bc}	4.59 ± 0.02^{b}	8.18 ± 0.03^{b}	10.42 ± 0.04^{b}
K_2SiO_3	2.94 ± 0.02^{a}	7.73 ± 0.02^{a}	8.18 ± 0.05^{a}	17.45 ± 0.04^{a}	32.55 ± 0.04^{a}
Water control	0.25 ± 0.02^{b}	0.70 ± 0.01^{c}	1.15 ± 0.02^{c}	1.75 ± 0.02^{c}	2.20 ± 0.02^{c}
Significance	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
CV (%)	27.94	26.50	14.07	12.71	16.82

HAI = hours after inoculation; CV = coefficient of variation;
Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 3 Expression of enzyme polyphenol oxidase (PPO) in glutinous rice plants after treating elicitors

Treatment	PPO activity at hours after inoculation (µg/protein/min)				
	0 HAI	3 HAI	6 HAI	9 HAI	12 HAI
$CaSiO_3$	847.92 ± 7.24^{b}	1062.50 ± 12.33^{b}	1277.08 ± 14.38^{b}	1585.42 ± 14.78^{b}	2164.58 ± 11.56^{b}
Na_2SiO_3	316.92 ± 3.58^{b}	764.58 ± 5.47^{b}	958.33 ± 5.74^{b}	1266.67 ± 11.48^{bc}	1687.50 ± 8.43^{bc}
K_2SiO_3	1347.92 ± 17.93^{a}	1918.75 ± 6.22^{a}	2535.42 ± 17.84^{a}	3495.83 ± 18.21^{a}	4297.92 ± 16.24^{a}
Water control	258.33 ± 5.11^{b}	475.00 ± 4.73^{b}	745.83 ± 9.37^{b}	910.42 ± 7.33^{c}	997.92 ± 3.79^{c}
Significance	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
CV (%)	15.39	29.48	25.66	17.60	18.59

HAI = hours after inoculation; CV = coefficient of variation;
Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

In silico binding of Si on phenylalanine ammonia-lyase based on docking studies

The three-dimensional structure was obtained using the MD method in the PROCHECK program with the Ramachandranplot server to predict possible bindings of Si on PAL in *Oryza sativa*. PAL binding sites were found by using the CASTp server and comparing it with the template. Designing, optimizing and docking of Si to the PAL binding region were performed using the GOLD 3.0.1 and Chemsketch software packages, respectively. The PAL - amino acids LYS45, LEU 56, and ARG66 played important roles in forming strong hydrogen bonds to Si (Fig. 1A). These results suggested that the binding of Si at the active site leads to the formation of an active complex with PAL.

In silico binding of Si on polyphenol oxidase based on docking studies

The predicted three-dimensional structure and MD, as well as validation using the Ramachandran plot server and PROCHECK program, were applied to find the possible binding sites of Si on PPO from *Oryza sativa*. The search for PPO binding sites was done by using CASTp 3.0 server and then comparing with the template (Tian et al., 2018). The design and optimization of Si were conducted using the Chemsketch 12.0 software (Phadke et al., 2021). Si was docked to the PPO binding region using the GOLD 3.0.1 software. The selected docked conformations of Si with the PPO binding site were found. The PPO amino acids ASP 86, PRO89, and ARG 103 formed strong hydrogen bonds with Si (Fig. 1B). These results suggested that the binding of Si at the active site leads to the formation of an active complex with PPO.

Analysis of salicylic acid accumulation

The effect was quantified of exogenous silicate elicitors on the SA concentration in glutinous rice plants. The results indicated that all treatments enhanced the SA concentration at 24 HAI, as shown in Table 4. At 0 HAI, the SA concentrations of elicitor treatments and water control were approximately 16.76–16.86 µg/g and not significantly different from each other. After 24 hr, the endogenous SA values were 30.14%, 27.13% and 24.82% in the K_2SiO_3, $CaSiO_3$ and Na_2SiO_3 treatment groups, respectively, which were significantly higher than for the water control (17.63%). Among the three elicitor treatments, K_2SiO_3 stimulated the glutinous rice plants to produce the highest endogenous SA, with an increase of 13.38%. The other two treatments ($CaSiO_3$ and Na_2SiO_3) increased by 10.27% and 8.01%, respectively (Table 4).

Table 4 Effectiveness of elicitors on salicylic acid (SA) concentration on glutinous rice leaf

Treatment	Endogenous SA (µg/g of fresh weight)		Increase in SA activity (%)
	0 HAI^2	24 HAI	
$CaSiO_3$	16.86±2.75	27.13±2.14^{ab}	10.27^b
Na_2SiO_3	16.81±1.17	24.82±2.36^b	8.01^b
K_2SiO_3	16.76±1.37	30.14±2.28^a	13.38^a
Water control	16.78±2.11	17.63±1.75^c	0.85^c
Significance	ns	< 0.05	< 0.05
CV (%)	8.30	9.58	18.22

HAI = hours after inoculation; CV = coefficient of variation;
Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

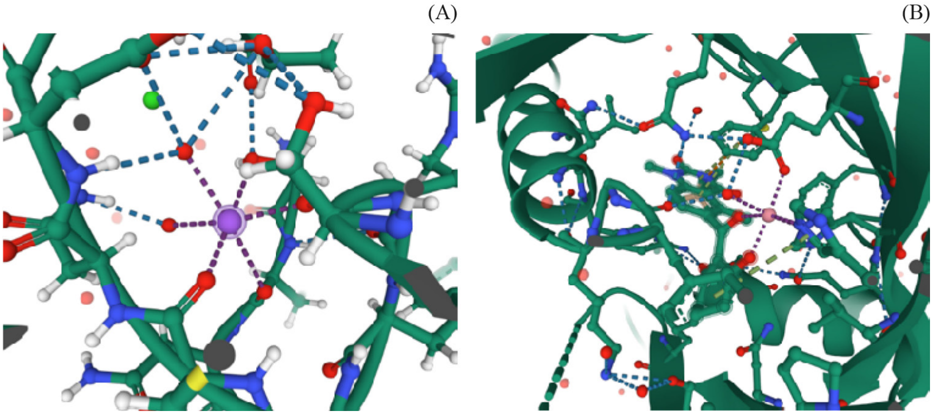

Fig. 1 (A) Molecular interactions of phenylalanine ammonia-lyase with Si (purple color), where hydrogen bonding shown by dotted red line, covalent bonding shown in dotted light blue lines, all hydrogen shown in white, oxygen atoms shown in red and carbon backbone in green; (B) molecular interactions of polyphenol with Si (orange color), where hydrogen bonding shown by dotted red line and covalent bonding shown by dotted blue line, all hydrogen shown in white, oxygen in red and carbon backbone in green

Effect of elicitors inhibiting Xoo density inside glutinous rice plants

The effects of the elicitors on the density of *Xoo* at 0 DAI, 7 DAI, 10 DAI, 20 DAI and 30 DAI are shown in Table 5. There was no *Xoo* colony at 0 DAI. At 7 DAI, the *Xoo* densities of treatments $CaSiO_3$ and K_2SiO_3 were 4.35×10^6 and 3.79×10^6 CFU/mL, respectively, which were both significantly lower than for Na_2SiO_3 (8.95×10^6 CFU/mL) or the water control (7.60×10^7 CFU/mL). At 10 DAG and 20 DAG, the *Xoo* densities of the Si treatments continued to be signifcantly lower than those of the water control. At 30 DAI, the colonies of *Xoo* had increased to 8.20×10^9 CFU/mL in the $CaSiO_3$ group, 8.39×10^{10} CFU/mL for Na_2SiO_3 and 7.46×10^9 CFU/mL for K_2SiO_3, which were significantly higher than for the water control (5.49×10^{14} CFU/mL), as shown in Table 5. The BLB lesions on the glutinous rice leaves of all treatments at 30 DAI are illustrated in Fig. 2.

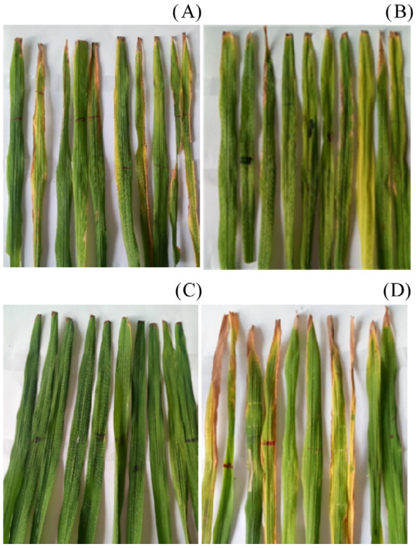

Fig. 2 Bacterial leaf blight lesions on glutinous rice leaves after treatment with Si at 30 d after inoculation for net-house conditions: (A) sprayed with $CaSiO_3$; (B) sprayed with Na_2SiO_3; (C) sprayed with K_2SiO_3; (D) water control, where leaves were tip-cut and dipped into prepared suspension of *Xoo*

Effect of elicitors against bacterial leaf blight in field conditions

For the field conditions, the effect of the elicitors against BLB was evaluated based on the growth and yield parameters (DS, amylose percentage and yield).

The disease severity of BLB for the field conditions is indicated on Table 6. At the 67 DAI, the DS levels of the treatments with $CaSiO_3$ and K_2SiO_3 were 0.43 and 0.77%, respectively, which were both significantly lower than for the Silimax treatment (positive control, at 1.28%), and the water control (negative control, at 3.18%). At 74 DAG, the DS levels for $CaSiO_3$ (32.49%) and K_2SiO_3 (34.78%) were not significantly different from Silimax (36.95%), although all were significantly lower than for the water control (46.10%). The treatment with Na_2SiO_3 had a DS level of 42.06%, which was not significantly different to the water control. At 81 DAG and 88 DAG, the DS levels for $CaSiO_3$, K_2SiO_3, and Silimax were not significantly different, having gains of 12.10–22.49% that were significantly lower than for Na_2SiO_3 (21.03–31.37%) and the water control (37.88–41.22%), as shown in Table 6.

K_2SiO_3 helped the glutinous rice plants to gain high levels of amylose at 4.88%, which was significantly higher than for Silimax (4.56%) and the water control (4.36%). The other elicitors ($CaSiO_3$ and Na_2SiO_3) had lower efficacy in increasing the amylose percentage (4.65–4.78%), which were not significantly different from Silimax, but were significantly lower than for the water control (Table 6).

The glutinous rice yields were in the range 6.25–6.46 t/ha for the K_2SiO_3 and $CaSiO_3$ elicitor treatments, which were not significantly different from Silimax (6.19 t/ha), but were significantly higher than for the water control (5.69 t/ha). The last elicitor treatment, Na_2SiO_3, yielded 6.07 t/ha, which was not significantly different from either the Silimax or the water control (Table 6).

Table 5 *Xoo* densities in glutinous rice leaves for net-house conditions

Treatment	*Xoo* density in glutinous rice leaves at days after inoculation (CFU/mL)				
	0 DAI	7 DAI	10 DAI	20 DAI	30 DAI
$CaSiO_3$	0.00	4.35×10^{6c}	8.43×10^{7b}	3.97×10^{8c}	8.20×10^{9c}
Na_2SiO_3	0.00	8.95×10^{6b}	2.58×10^{8b}	5.57×10^{8b}	8.39×10^{10b}
K_2SiO_3	0.00	3.79×10^{6c}	4.23×10^{7c}	3.81×10^{8c}	7.46×10^{9d}
Water control	0.00	7.60×10^{7a}	5.64×10^{8a}	7.76×10^{10a}	5.49×10^{14a}
Significance		< 0.05	< 0.05	< 0.05	< 0.05
CV (%)		0.78	2.67	0.69	0.28

DAI = days after inoculation; CV = coefficient of variation

Mean in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 6 Disease severity of bacterial leaf blight, amylose percentage and yield for field conditions

Treatment	Days after growing				Amylose percentage (%)^1	Yield (t/ha)
	67	74	81	88		
$CaSiO_3$	0.43 ± 0.01^d	32.49 ± 1.35^c	18.23 ± 1.28^c	12.10 ± 1.03^c	4.78 ± 0.03^{ab}	6.25 ± 0.27^a
Na_2SiO_3	2.77 ± 0.03^a	42.06 ± 1.68^{ab}	31.37 ± 1.99^b	21.03 ± 2.67^b	4.65 ± 0.05^{ab}	6.07 ± 0.95^{ab}
K_2SiO_3	0.77 ± 0.01^c	34.78 ± 2.15^{bc}	22.49 ± 2.38^c	14.35 ± 1.05^c	4.88 ± 0.03^a	6.46 ± 0.81^a
Silimax	1.28 ± 0.01^b	36.95 ± 2.36^{bc}	21.52 ± 2.15^c	15.15 ± 1.07^c	4.56 ± 0.03^{bc}	6.19 ± 0.37^a
Water control	3.18 ± 0.02^a	46.10 ± 1.97^a	41.22 ± 1.35^a	37.88 ± 2.17^a	4.36 ± 0.03^c	5.69 ± 0.84^b
Significance	< 0.01	< 0.05	< 0.01	< 0.01	< 0.05	< 0.05
CV (%)	10.84	13.30	9.35	8.57	2.26	4.25

CV = coefficient of variation

Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Discussion

The *Xoo* could swim and penetrate glutinous rice plants through the stomata on the leaf surface and hydathodes at the leaf margin. After successful infection, the *Xoo* remained and multiplied in the intercellular spaces of the glutinous rice cells, continuing to enter cells or spreading to the whole plant through the xylem. After a few days, it reached a high density and caused blight lesions on the leaves. The pathogenic *Xanthomonas* (*Xoo* specifically) use secretion system type III to infect and multiply inside plant tissues (Ma et al., 2020). The silicate elicitor could be applied to induce resistance mechanisms in glutinous rice plants against various diseases and their yield.

After *Xoo* invasion and colonization, the defense of Si-induced glutinous rice plants may have involved enzyme expression of PAL and PPO and the production of endogenous salicylic acid (SA). In the present study, analysis of PAL activity showed that at 12 HAI, the treatments of $CaSiO_3$, Na_2SiO_3 and K_2SiO_3 had significantly higher PAL than that of the water control. These results agreed with the research of Nativitas-Lima et al. (2021), who applied K_2SiO_3 to manage the fungus *Monilinia fructicola* in peaches. In date palm, *Bifurcaria*-extracted polysaccharide could induce PAL activity in the roots at levels five times higher than in the water control (Bouissil et al., 2022). The induction pattern of the defense enzyme PAL showed maximum activity at 12 HAI in tomato plants against the bacterium *Ralstonia solanacearum* (Vanitha et al., 2009). Similarly, PPO is another critical enzyme of the phenylpropanoid pathway responsible for producing polyphenols (Dixon et al., 2002; Sharma et al., 2019). The present study showed that the treatments of $CaSiO_3$, Na_2SiO_3 and K_2SiO_3 had signifcantly higher PPO concentrations than the water control. Similarly, the results of Nativitas-Lima et al. (2021) showed an increase by 167% in the PPO activity after applying K_2SiO_3 in peach against *Monilinia fructicola*. In addition to the correlation of the PAL and PPO activities with decreased and increased levels of the polyphenols underlying pathogen infection, invasion and colonization, each enzyme could have its specific role. PPO has an anti-nutritive effect on phytopathogens (Vanitha et al., 2009). Furthermore, PPO could oxidize the less toxic phenolic compounds to highly toxic quinones, so it plays a vital role in directly inhibiting phytopathogens (Lavanya et al., 2022). PAL is responsible for the biosynthesis of SA, an important defense signal in the plant's systemic resistance (Kim and Hwang, 2014). SA is the systemic signal underlying induced resistance in the interaction of Si application and biotroph pathogen infection (Wang et al., 2017). In *Arabidopsis*, Si application induces SA-dependent resistance, expressed by a slight increase in the SA concentration in resistance against powdery mildew (Wang et al., 2020). The results of endogenous SA accumulation in the present study were consistent with those of Wang et al. (2020) in *Arabidopsis*.

In Si-treated glutinous rice plants, the interaction mechanism between the silicate elicitor and PAL or PPO has not been revealed or understood in former studies, nor has it induced resistance. Therefore, knowledge of the interaction of the silicate elicitor and PAL/PPO could contribute to explaining the induced resistance mechanism in glutinous rice. Generally, the algorithm side-chain fluctuations and inflexible docking have been evaluated, and multiple compounds confirmed, which were used to obtain a suitable docking complex. The present research showed that the amino acids LYS45, LEU56 and ARG66 could be active sites of PAL, and the amino acids ASP86, PRO89, and ARG103 could be active sites of PPO. These active sites of PAL and PPO form strong hydrogen bonds with the exogenous silicate elicitor, forming a complex with the active parts of PAL and PPO. This activates PAL and PPO during induced resistance. The present results were similar those of Thepbandit et al. (2021), who found that the α-helix, β-sheet and other secondary conformations of the structural β-sheet on PR1b were active sites, combining with the exogenous elicitor of SA to form resistance to BLB in rice plants.

Following the defense mechanism of the elicitor Si, the density of *Xoo* inside the glutinous rice vascular system could provide another aspect of efficiency after silicate application. Compared with the water control treatment, the low *Xoo* densities of the Si-induced treatments proved that the defense mechanism inside glutinous rice plants was efficiently activated. As a consequence, the BLB lesions that developed were short and minor. The results of the Si elicitor in the present study were consistent with the results of Le Thanh et al. (2017) when they applied an exogenous SA elicitor in rice plants.

The efficacy of Si application to BLB was evaluated in field conditions. In general, $CaSiO_3$ and K_2SiO_3 had high efficacy, equivalent to the positive control. The next most effective was Na_2SiO_3, as shown through the DS parameter levels at different observation times. In field conditions, Si was foliage-sprayed at 25 DAG and 45 DAG in its salt forms of $CaSiO_3$, Na_2SiO_3 and K_2SiO_3, after which their effect on managing BLB was evaluated. The results showed that at 88 DAG, the DS levels of $CaSi0_3$, Na_2SiO_3 and K_2SiO_3 were approximately 12.10–21.03%, corresponding to a reduction of 16.85–25.78% compared to the control. These results were in line with studies by Zhang et al. (2012) and Zhan et al. (2018) in rice plants, Wang et al. (2020) in *Arabidopsis* and Feng et al. (2021) in rapeseed. Applying Si at 1.5 mM reduced the DS level of sheath blight by 2.96 units in the sensitive rice cultivar Ningjing 1 (Zhang et al., 2012). Furthermore, root application of Si was reported to elevate induced resistance against rice root-knot disease (Zhan et al., 2018). Si also induced disease resistance in *Arabidopsis* against powdery mildew (Wang et al., 2020). Feng et al. (2021) conducted field experiments in two crop seasons where rapeseed plants were treated with K_2SiO_3 at 10 mM against *Sclerotinia* stem rot and showed that the application of the Si elicitor reduced disease incidence. This result was consistent with the efficacy of Si in the present research. The percentage of amylose content is usually in the range 2–18% (Masniawati et al., 2018). Glutinous rice is classified according to whether the percentage of amylose is good (0–5%), very low (>5–12%), or low (>12–20%) (Phuwadolpaisarn, 2021). The amylose percentage was under 5% in the present study, indicating it was good, according to Phuwadolpaisarn (2021). The present results indicated that at 88 DAG, the DS levels of $CaSi0_3$ and K_2SiO_3 were approximately 12.10–14.35%, which were significantly lower than for Na_2SiO_3 (21.03%) and the water control (37.88%). These pathogenesis results correlated with increasing the yield of glutinous rice. The yields of two of the elicitor treatments ($CaSiO_3$ and K_2SiO_3) were 6.25–6.46 t/ha, significantly higher than for the water control (5.69 t/ha). The third elicitor treatment (Na_2SiO_3) had a yield of 6.07 t/ha, which was not significantly different from the water control. The results showed that after Si application, the health of the glutinous rice plants was good, so the plants could resist *Xoo* invasion and produce high yields. In addition, the results showed that $CaSi0_3$ and K_2SiO_3 were more effective sources of applied Si than Na_2SiO_3. The increasing yield in glutinous rice grains in the present study was similar to that of Ligaba-Osena et al. (2020), who showed that an application of Na_2SiO_3 increased the biomass and yield of tef (*Eragrostis tef* (Zucc.) Trotter).

Silicate application in the forms of $CaSiO_3$, K_2SiO_3 and Na_2SiO_3 helped the glutinous rice plants resist BLB in field conditions and improved disease resistance mechanisms including SA accumulation and the upregulation of the PAL and PPO defense enzymes. Considering the structure of PAL, the amino acids LYS45, LEU56 and ARG66 could be its active sites in forming strong hydrogen bonds with exogenous silicate elicitors. These form a complex with the active residues of PAL, contributing to disease resistance. Considering the structure of PPO, the amino acids ASP86, PRO89, and ARG103 could be active sites, playing important roles similar to LYS45, LEU56, and ARG66 with PAL. In summary, applying Si increased the density of *Xoo*, maintained amylose percentages and increased yield. Among the types of silicon, K_2SiO_3 and $CaSiO_3$ showed higher efficacy than Na_2SiO_3 in glutinous rice plants. Based on the known current published literature, the present report is the first showing the beneficial efficacy of Si in glutinous rice plants. Further research will conduct a risk assessment, based on the lethality of K_2SiO_3 and $CaSiO_3$ to aquatic species (fish) and mammals (rat). In addition, 6–15 field trials will be established involving K_2SiO_3 and $CaSiO_3$ in different provinces in Vietnam in different growing seasons. This additional information will be used to register the Si product as a commercial pesticide.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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