

Silicon Root Irrigation Enhances Barley Resistance to *Fusarium* Head Blight

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Silicon (Si) is recognized for its protective role in decreasing disease damage when absorbed by barley plants and has been proposed as a possible solution against *Fusarium* head blight, associated with devastating agronomic effects on overall yield and grain quality. However, root treatment of exogenous Si irrigating to enhance host resistance to *Fusarium* infection is unknown. For this purpose, a series of greenhouse experiments was conducted to examine the effects of Si irrigation at 1.7 mM to roots on pathogen development in barley heads. Two barley cultivars with contrasting FHB resistance (moderately resistant Arabi Aswad, AS, and moderately susceptible Arabi Abiad, AB) and infected with four *Fusarium* species with diverse pathogenicity were used. The quantification of the disease was through the determination of the disease incidence (DI, Type I resistance), disease severity (DS, Type II) and area under disease progress curve (AUDPC) calculated on the basis of DI and DS. Si absorption in barley enhanced the defense system in head tissues to pathogen invasion; FHB developed more severely on AS and AB plants grown without Si irrigation than on plants supplied with Si. Barley plants treated with exogenous Si irrigating were associated with a reduction of up to 19.3%, 19.8%, 18.7%, and 20.0%, respectively, in DI, DS and AUDPC calculated on the basis of DI and DS. Si contributed to the reduction of FHB in barley, especially for the moderately resistant cultivar; however, Si reduced the intensity of FHB in AB to a level comparable with AS. Importantly, Si treatment at 1.7 mM decreased disease damage FHB in previous bio-trials conducted on AS and AB under *in vitro* and field environments, showing that Si enhanced the expression of resistance to FHB infection in seedlings and adult barley plants. Taken together, the link of Si and host resistance provided a greater decrease in head blight in which both cultivars had augmented performances upon exogenous Si irrigating to roots; highlighting that Si is a potential safe and efficient policy to defend barley when invaded by *Fusarium*.

Key words: barley resistance, *Fusarium* species, *Hordeum vulgare* resistance, integrated disease management, silicon root irrigation.

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated grain crops ranking fourth in importance, among small-grain cereals, and successfully cultivated under a board range of climates. From an economic point of view, its importance arises from being largely utilized for feed and food production worldwide. Almost 80%–90% of *H. vulgare* grain production is appropriated for livestock feed, while the remaining 10% is transformed into distilling, baking and malt for brewing (FAW, 2022). Its yield is directly menaced by diseases, such as *Fusarium* head blight (FHB), one of the most common and noxious fungal diseases of barley (Ogrodowicz *et al.*, 2020). A pathogen complex of several *Fusarium* species can cause blight in head tissues (Fernando *et al.*, 2021); the most globally pathogenic and distributed causal agents encompass *F. graminearum* and *F. culmorum* (Inbaia *et al.*, 2023). FHB leads to premature brownish discolouration of florets scattered throughout the spike, which results in the formation of discoloured grains (brown, pink, red, orange or tan) (Dahl and Wilson, 2018). It results in considerable losses in crop production, quality and safety due to the risk of kernel contamination by a large variety of mycotoxins (Janssen *et al.*, 2018).

Breeding resistant *H. vulgare* cultivars is an efficient policy to defeating FHB. Head blight resistance of barley (Sakr, 2022a) is a quantitative trait expressed from several quantitative resistance loci and linked with two types: resistance to floret infection (Type I) and spreading of the infection within the head (Type II) (Dahl and Wilson 2018; Janssen *et al.*, 2018). However, commercial cultivars resistant to *Fusarium* development in head tissues are not yet obtainable to growers due to FHB resistance of *H. vulgare* is complex to use (Ogrodowicz *et al.*, 2020). Furthermore, efficient chemical management of FHB resulting in potential environmental damage and significant economic losses is restricted to a small time window at around anthesis, when primary head invasion generally occurs (Janssen *et al.*, 2018), and hence augmenting complexity of effective FHB control (Fernando *et al.*, 2021). In novel years, the issue of FHB-barley has been further complicated by important interactions among

management techniques, pathogenicity of diverse *Fusarium* organisms and continual extreme climatic events (Sakr, 2022a). Hence, an alternative eco-friendly policy must be recognized in the near future to reduce damages due to FHB (Dahl and Wilson, 2018).

A progressively common policy is to increase the defense of plants against pathogens. In this scenario, the most promising approach is the use of silicon (Si) fertilization as part of an integrated disease management approach (Debona *et al.*, 2017). In spite of Si has not been referred a major element for higher plants (Kaur and Greger, 2019), it has been shown to be helpful for the healthy development and growth of several plants exposed to biotic and abiotic constraints (Guo-Chao *et al.*, 2018). The existence of soluble Si in plant shoots has been proven to decrease the intensity of several economically important fungal diseases (Deshmukh *et al.*, 2016). The action mechanism of Si in host defense is linked with three actions, viz., the physical, biochemical, and molecular action of mechanisms (Debona *et al.*, 2017), identified as formation of a silicon-cuticle double layer, cell wall stiffness reinforcement, callose deposition, papillae formation, promoting host biochemical defenses via accumulation of hydrogen peroxide at infection sites, accumulation of antimicrobial compounds (pathogenesis-related proteins, phytoalexins, phenylpropanoids, and flavonoids), enhanced activities of defense enzymes, gene expression induced by stressors in plants, and signal transduction (Guo-Chao *et al.*, 2018; Kaur and Greger, 2019). In plants, the terminal Si level depends on various factors, encompassing host species, host age, the cultivar's ability to absorb and distribute Si around the plant, as well as the content of Si obtainable in soil solution (Deshmukh *et al.*, 2016).

H. vulgare is recognized as a Si-absorber and absorb relatively high quantities of Si in shoots and leaves (Guo-Chao *et al.*, 2018; Kaur and Greger, 2019). Fungal diseases of barley including powdery mildew (*Blumeria graminis* f. sp. *hordei*) and spot blotch (*Bipolaris sorokiniana*) have been reported to be decreased by Si application through a better performance of the primary metabolism of *H. vulgare*

plants (Wiese *et al.*, 2005; Holz *et al.*, 2022). In recent years, Sakr (2021b) reported encouraging findings with Si and FHB-barley; Si decreased the invasion rate and *Fusarium* spreading in barley head tissues by increasing Type I and Type II under controlled conditions. A little success was obtained with soil amendments than with foliar applications, showing that Si rates augmented in plants fed Si via the roots and leaves (Sakr, 2021b). Interestingly, root irrigating with Si has proven to reinforce the root growth resulting in its enhanced capacity of Si absorption and a better resistance to fungal infection in some pathosystems, i.e., *Blumeria graminis* f.sp. *tritici* causing wheat powdery mildew, *Magnaporthe oryzae* causing rice blast, *Rhizoctonia solani* causing rice sheath blight, *Phakopsora pachyrhizi* causing Asian soybean rust (Zhang *et al.*, 2003; Guevel *et al.*, 2007; Arsenault-Labrecque *et al.*, 2012; Du *et al.*, 2022) including a decrease of FHB development on wheat (Sakr, 2022b; Sakr and Kurdali, 2023b). Thus, researches on whether exogenous Si irrigating to roots could have a positive function on *H. vulgare* resistance to *Fusarium* invasion in head tissues appears completely necessary but are still unknown.

Si advantage is crucial for sustainable production of barley (Sakr, 2021b), taken into account the worldwide dominance of *Fusarium* species (Ogrodowicz *et al.*, 2020; Fernando *et al.*, 2021). To our knowledge, this is the first report to explore the effect of Si irrigating via the root system on enhancing the components of barley resistance to FHB invasion under conditions controlling strictly all biotic and abiotic factors. Disease incidence (DI, Type I resistance), disease severity (DS, Type II) and Area Under Disease Progress Curve (AUDPC) calculated on the basis of DI and DS were evaluated in two barley cultivars with different FHB resistance levels supplied or not with Si. The ability of Si to enhance resistance on moderately susceptible to a level comparable to moderately resistant not amended with Si was also investigated. In addition, the relationships were examined between the current findings and the damage of FHB in previous analyses conducted on barley cultivars under *in vitro* and field environments to check whether Si could increase the expression of resistance

to FHB infection at the earliest and latest barley development stages during FHB infection.

MATERIALS AND METHODS

Barley materials and growth conditions

Two barley cultivars of Syrian origin: Arabi Aswad (AS, moderately resistant) and Arabi Abiad (AB, moderately susceptible), pre-selected according to different resistance to FHB as assessed in inoculation trials under *in vitro*, controlled and field conditions (Sakr 2023a), were used in the experiments. AS and AB were selected due to their identical anthesis and maturity dates, and board usage in the market (Ceccarelli *et al.*, 1987). Prior to being utilized in trials, AS and AB seeds were surface sterilized by immersion in 0.5% sodium hypochlorite solution (NaOCl) for 1 minute. The seeds were then rinsed three times in sterile water. Eight surface-sterilized barely seeds were sown in plastic pots (20 × 15 cm) containing 2 kg of pasteurized soil. Using a gamma irradiator (ROBO, Russia), the pasteurization of the soil was carried out at 5 k Gy of Gamma Ray with Co⁶⁰ source. The soil used was air dried and sieved (3 mm). The physicochemical characteristics of the clay soil (57% clay, 39% loam, 2% sand) used in the study were as follows: organic matter of 1.25%; K, Na, Ca, Mg = 1.81, 2.99, 33.1, 14 mg/100 g soil respectively; P = 13.4 mM and pH 7.8. After seedling emergence, they were thinned to five plants per pot, and nitrogen, in the form of urea, was applied at 0.173 g/pot at two dates: emergence and tillering.

Throughout the experiment, the plants were kept under controlled climatic conditions in a growth chamber with air conditioning for temperature control. Average daily temperature was set to 20°C day/night, 16 h of per day with a relative air humidity of 60%.

Inoculation procedure

Till now, the recovery of head blight pathogens has not been achieved from Syria barley plants. Nevertheless, *Fusarium* species are frequently recovered from naturally infected wheat fields and known to strongly infect barley spikes (Sakr, 2023a). All *Fusarium* isolates, including six *F. solani*, five *F. culmorum*, four *F. verticillioides* (synonym *F. moniliforme*) isolates and one *F. equiseti* isolate, were

isolated from naturally infected wheat grain. The 16 isolates were monosporic derived cultures of the original field isolates and were selected for their contrasting pathogenicity based on previous several experimental observations (Sakr, 2022a, 2023a). On Petri dishes with potato dextrose agar (PDA) with 13 mg/l kanamycin sulphate added after autoclaving, the isolates were morphologically identified with the aid of the Leslie and Summerell (2006) manual on the basis of microscopic studies of the shape and size of macro- and micro-conidia, and were molecularly distinguished by *Random amplification of polymorphic DNA* markers (Sakr, 2023a). These 16 *Fusarium* isolates were used in previous experiments conducted under *in vitro*, growth chamber and field trials to assess resistance levels of AS and AB (Sakr, 2022a, 2023a), and resistance levels were correctly and precisely distinguished.

Single spore isolates were stored short term on PDA at 4°C and long term by freezing at -16°C or in sterile distilled water at 4°C, and fresh cultures were produced on PDA medium. After 14 days, conidia were collected in sterile distilled water (SDW). Then, the suspensions were filtered through two layers of sterile cheesecloth to remove agar and adhering mycelia. The spore concentration was adjusted prior to use with the aid of a Neubauer chamber under an optical microscope and diluted to 5×10^4 conidia/ml as inoculum sources.

Disease evaluation

The quantification of the disease resistance in AS and AB separately inoculated with the 16 *Fusarium* cultures was through the determination of the incidence of FHB (DI, Type I resistance), severity of FHB (DS, Type II) as well as area under disease progress curve (AUDPC) calculated on the basis of DI and DS. At full flowering (GS=65) and by using a conidial suspension for the 16 *Fusarium* isolates, barley plants were spraying inoculated on the whole head for bleaching of spikes (DI) or by inoculation in the central floret of the head (single flower inoculation) for bleaching of spikelets (DS). For the single spikelet inoculation method, 10 µl of inoculum suspension was injected between the palea and lemma in the central floret of the head, utilizing a sterile micropipette tip. Each isolate was used independently (not mixed). Non-inoculated AS and AB

plants were sprayed with SDW and exposed to the same conditions as the inoculated plants. The both types of inoculations, head and floret, were made on the discrete heads of the same cultivar in two separate experiments. Regardless of inoculation method, immediately after inoculation, plants were maintained inside plastic bags the inner surfaces of which had been sprayed with SDW. AS and AB plants were kept under these conditions for 48 h in order to provide humid conditions favorable for the initial phase of pathogenesis.

DI, DS and AUDPC calculated on the basis of DI and DS were determined to decide the degree of *Fusarium* infection in light of visual damages in head tissues. DI was measured by counting the number of heads with obvious FHB symptoms (i.e., partially or fully bleached heads) and was expressed as the percentage of barley heads with symptoms. FHB severity was expressed as percentage of spikelets with clear FHB symptoms in comparing with the total number of spikelets per head as described in Sakr (2023a). AUDPC for each plant in each treatment and experiment was calculated on the basis of DI and DS using the trapezoid integration of the disease progress curve (Jeger *et al.*, 2001) over the progressive blighting of heads scored at 7, 14, 21 and 28 days post inoculation. AUDPC was conducted with the beginning of heads with discoloured spikelets that are indicative of head blight about 1 week after inoculation.

Silicon application

Pure Si was employed to avoid the confounding impact of other nutritive elements in some Si-based soil amendments (Deshmukh *et al.*, 2016). The silicon source was an analytically pure SiO₂ powder (H₄SiO₄, Kieselsaure, Carl Roth GmbH + Co. KG, composed by a minimum silicon content of 99% Si), which was supplied along with the irrigation solution. In a previous study conducted on barley (Sakr 2021b), a SiO₂ powder decreased FHB damage in head tissues expressed by DI and DS of pathogenic *Fusarium* isolates following artificial spike and spikelet inoculation under controlled conditions (Sakr, 2021b), therefore, SiO₂ powder was preferred as Si source in the current research. Si as irrigation solution was prepared by dissolving a SiO₂

powder in demineralized water. From the sowing till the fungal spraying time, the barley plants were weekly irrigated with 300 ml (per pot) with a concentration of 1.7 mM Si. The control was irrigated with 300 ml of SDW. AS and AB were watered with the similar volume of irrigation solution in the existence of Si (1.7 mM Si) or not.

Experimental design

The trials were carried out to correctly and precisely assess the influence of root treatment of exogenous Si irrigating into AS and AB on head blight DI, DS and AUDPC calculated on the basis of DI and DS (Figure 1). A $2 \times 2 \times 16$ factorial experiment, consisting of two Si concentrations (0 and 1.7 mM, referred to as –Si and +Si plants thereafter), two barley cultivars with different resistance levels: AS and AB, and 16 *Fusarium* isolates causing FHB, were arranged in a randomized design with three replications. The experiment was repeated twice.

Si ability to enhance barley resistance under several experimental conditions

The bio-efficacy of Si to increase AS and AB barley resistance to FHB disease was measured by latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile length reduction (CLR) of a coleoptile infection under *in vitro* conditions (Sakr, 2023b) (Table 1). The reduction in head blight symptoms due to the effect of Si was also expressed under field conditions as DI (Type I resistance), DS (Type II) and *Fusarium*-damaged kernels (Type III) (Sakr and Kurdali, 2023a) (Table 1). Therefore, we were able to examine the relationships between the current findings with the previous results of *in vitro* and field environments to check whether Si could increase the expression of resistance to FHB infection at the earliest and latest barley development stages during FHB infection.

Statistical analyses

Prior to ANOVA, the percentages of DI, DS and AUDPC calculated on the basis of DI and DS were arcsine angular transformed to stabilize variances. Data were subjected to factorial analysis of variance

(ANOVA) and the means of treatments compared by Fisher's least significant difference test at $p < 0.05$ using the DSASTAT add-in version 2011. Single degree-of-freedom contrasts test was used to make comparisons between AB supplied with Si and AS non-supplied with Si. Comparison among FHB isolates infected AS and AB upon Si treatment was made by the contrast procedure.

RESULTS

Si application reduced disease development of *Fusarium* pathogens in barley

ANOVA showed that Si, cultivar, cultivar \times Si interaction had highly significant effects ($p \leq 0.05$) on DI, DS as well as AUDPC calculated on the basis of DI and DS (data not shown). On AS and AB, Si resulted in lower disease intensity based on the percentage of diseased florets and smaller necrotic patches, and less bleaching of the florets and discoloured kernels as compared to fungal-inoculated-controls (Figure 2). Regardless of botanical and pathogenic background for the host and fungal materials in the present research, FHB DI, DS and AUDPC calculated on the basis of DI and DS (Figure 3) were significantly decreased in +Si plants AS and AB barley plants inoculated with all analyzed *Fusarium* isolates relative to –Si plants, this indicates that root Si irrigation (1.7 mM) protected any tested barley cultivar whatever its quantitative resistance levels from infestation with any FHB pathogen whatever its pathogenic level under controlled environmental conditions. –Si barley plants supplied with SDW did not show diseased symptoms. In plants supplied with Si, DI, DS and AUDPC calculated on the basis of DI and DS were 19.3%, 19.8%, 18.7%, and 20.0%, respectively, smaller than in –Si barley plants. Si absorption in barley enhanced the defense system in head tissues to pathogen invasion; FHB developed more severely on AS and AB plants grown without Si irrigation than on plants supplied with Si. AS was significantly more resistant to head blight infection than AB (Figure 3).

Si (1.7 mM)-supplied cultivars, AS which is moderately resistant and AB which is moderately susceptible, had a DI that was lower by 23 and 14%, respectively in experiment 1; 22 and 19%, respectively in experiment 2, 20 and 18%, respectively in experiment 3 than plants without Si (Figure 3, A).

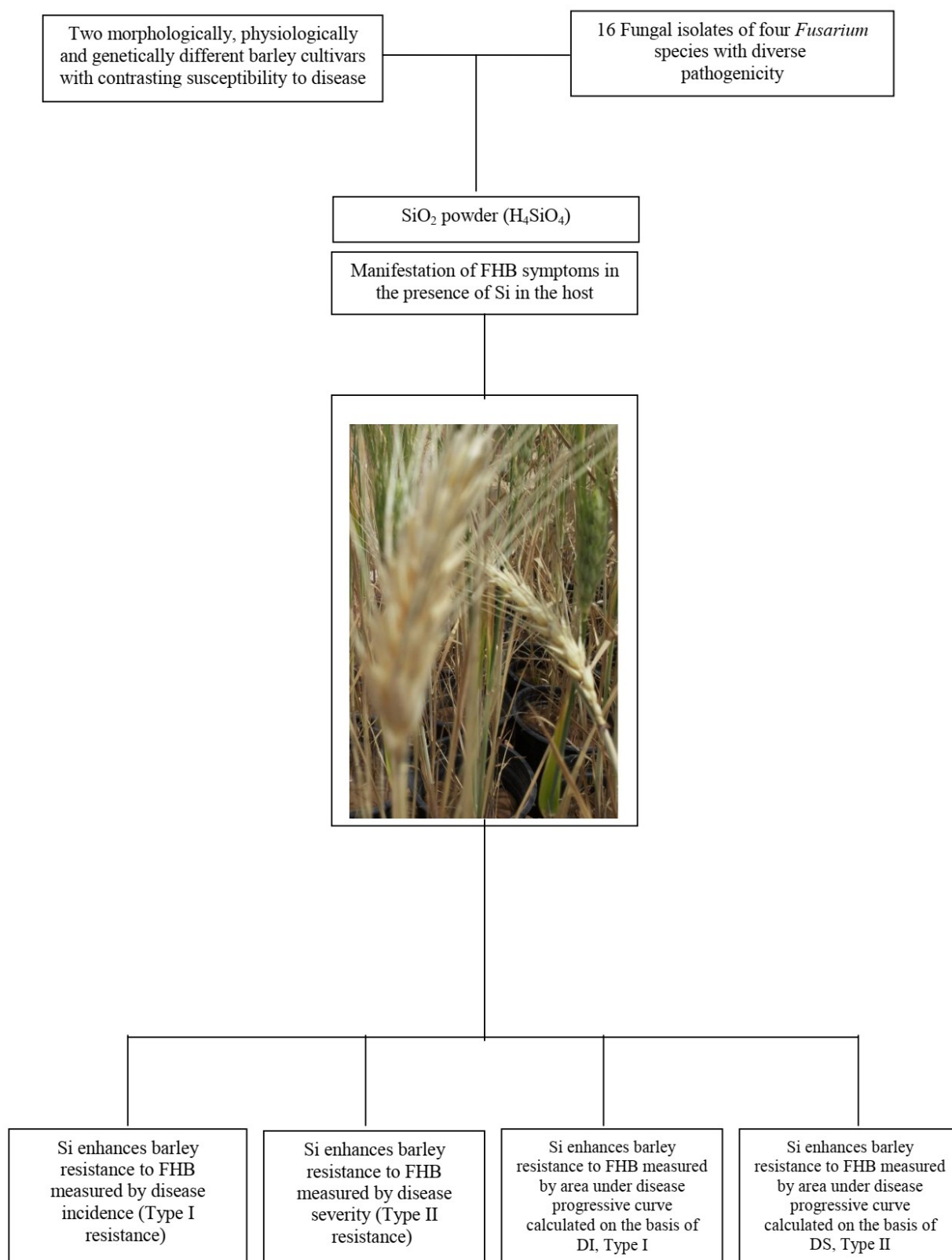


Figure 1. A schema of the experimental design in the study

Table 1: Influence of silicon at 1.7 mM in barley (Arabi Aswad, AS and Arabi Abiad, AB) resistance to Fusarium head blight measured by latent period (LP, days), area under disease progress curve (AUDPC) and coleoptile length reduction (CLR, %) under in vitro conditions, disease incidence (DI, Type I resistance), disease severity (DS, Type II resistance) and *Fusarium*-damaged kernels (FDK, Type III resistance) under field conditions

LP						
cultivars	Experiment 1		Experiment 2		Experiment 3	
	- Si	+ Si	- Si	+ Si	- Si	+ Si
AS	5.9b	6.9a	5.7b	6.7a	5.6b	6.7a
AB	6.8b	8.6a	6.9b	8.6a	6.8b	8.5a
AUDPC						
cultivars	Experiment 1		Experiment 2		Experiment 3	
	- Si	+ Si	- Si	+ Si	- Si	+ Si
AS	0.40a	0.34b	0.41a	0.35b	0.41a	0.35b
AB	0.34a	0.27b	0.32a	0.26b	0.33a	0.26b
CLR						
cultivars	Experiment 1		Experiment 2		Experiment 3	
	- Si	+ Si	- Si	+ Si	- Si	+ Si
AS	42a	35b	40a	34b	41a	35b
AB	33a	26b	32a	26b	31a	25b
DI						
cultivars	Experiment 1		Experiment 2		Experiment 3	
	- Si	+ Si	- Si	+ Si	- Si	+ Si
AS	38a	30b	33a	31b	40a	31b
AB	46a	39b	47a	40b	48a	40b
DS						
cultivars	Experiment 1		Experiment 2		Experiment 3	
	- Si	+ Si	- Si	+ Si	- Si	+ Si
AS	31a	24b	29a	23b	31a	24b
AB	39a	32b	38a	31b	40a	32b
FDK						
cultivars	Experiment 1		Experiment 2		Experiment 3	
	- Si	+ Si	- Si	+ Si	- Si	+ Si
AS	38a	31b	38a	30b	39a	30b
AB	39a	31b	38a	31b	40a	32b

According to the Fisher's LSD test, means followed by the same letter within a lineage are not significantly different at $p < 0.05$. Response measured by LP, AUDPC, CLR, Type I, Type II, Type III were presented by Sakr (2023b) and Sakr and Kurdali (2023a). A barley cultivar with higher value of LP and lower value of AUDPC, CLR, DI (Type I resistance), DS (Type II) and FDK (Type III resistance) was considered as more resistant than a barley cultivar with lower value of LP and higher values of AUDPC, CLR, DI (Type I resistance), DS (Type II) and FDK (Type III resistance).

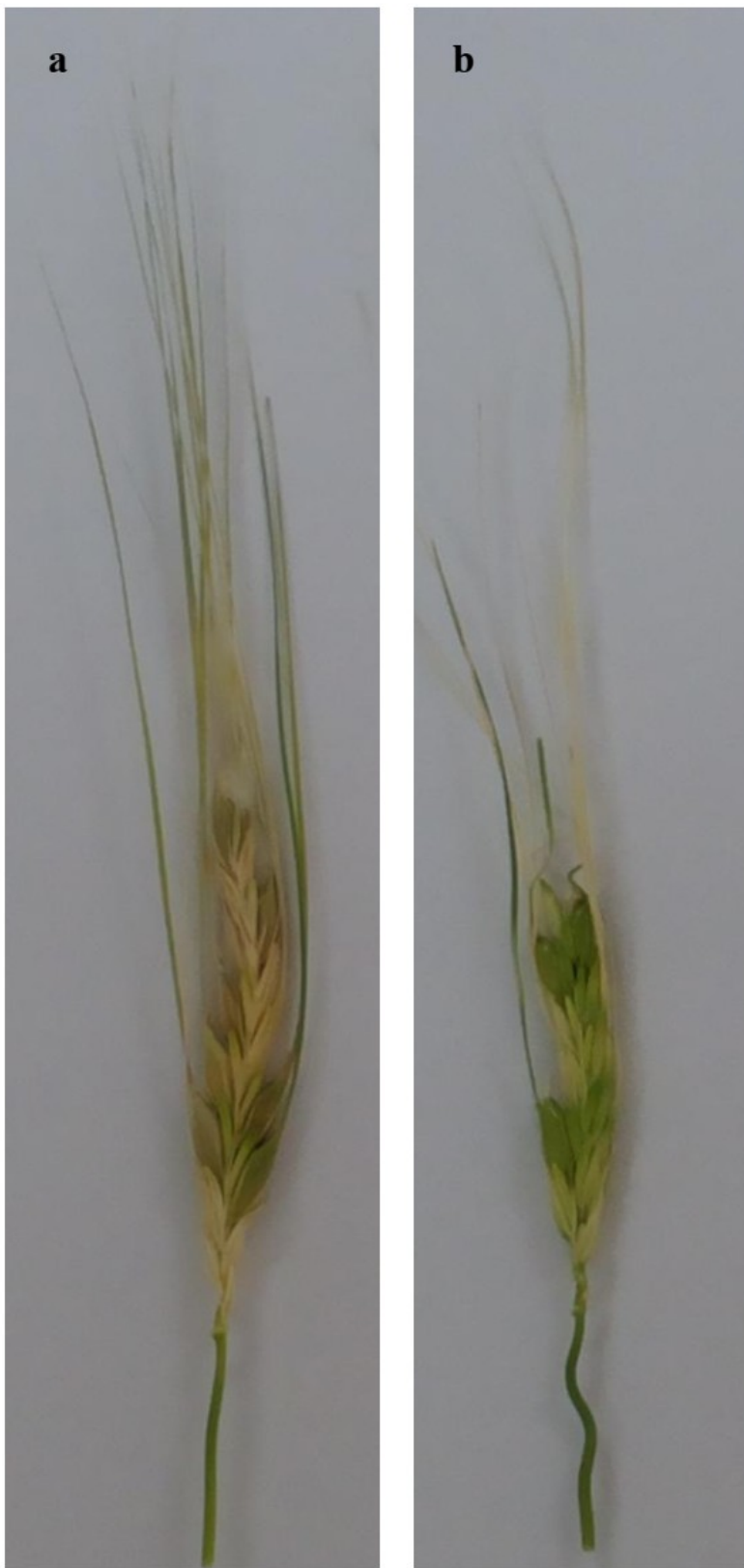


Figure 2. Root treatment of exogenous silicon irrigating at 1.7 mM enhances barley resistance to Fusarium head blight. FHB disease suppression on Arabi Abiad barley heads in response to silicon irrigation to soil at 21 days post inoculation under controlled conditions; (a) a barley head inoculated with FHB pathogen using an artificial head inoculation assay and no silicon application for root treatment, and (b) a barley head inoculated with FHB pathogen using an artificial head inoculation assay and silicon root irrigation at 1.7 mM

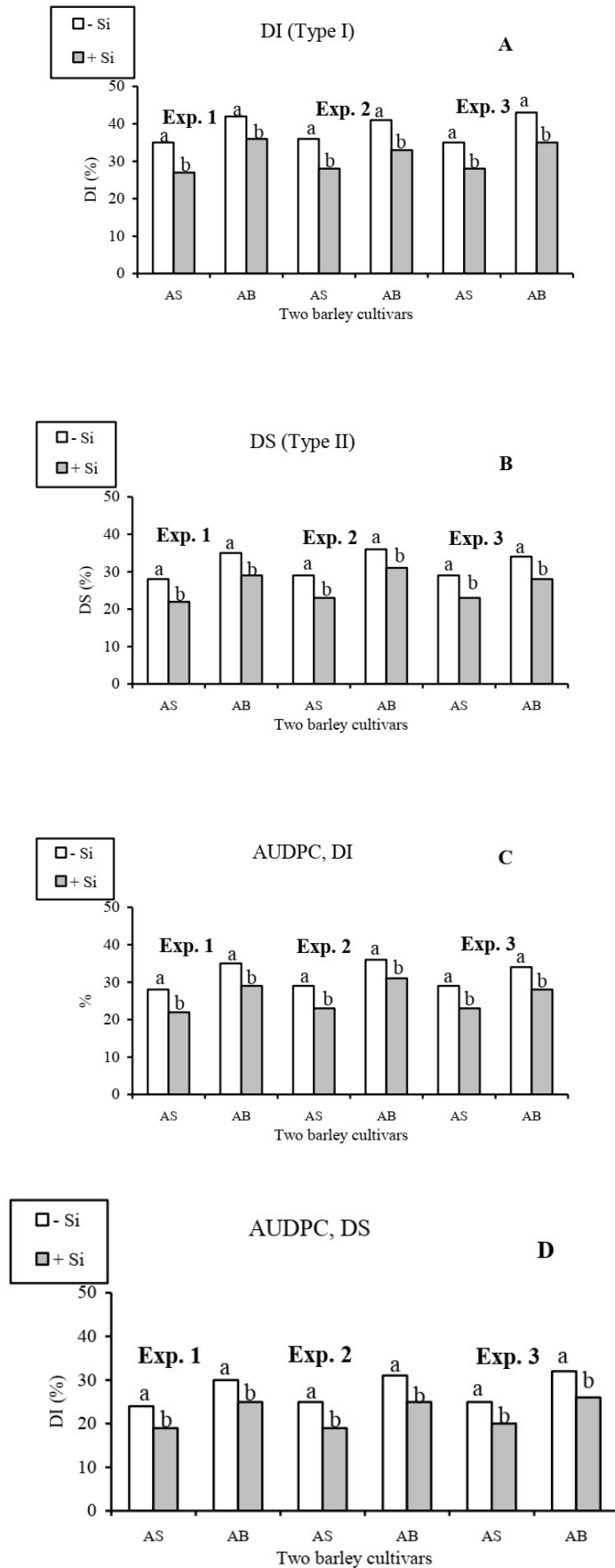


Figure 3. Influence of root treatment of exogenous silicon irrigating at 1.7 mM via roots in barley (Arabi Aswad, AS and Arabi Abiad, AB) resistance to Fusarium head blight measured DI (Type I resistance, Fig 3, A), DS (Type II, Fig 3, B) and area under disease progressive curve (AUDPC) calculated on the basis of DI, Type I and DS, Type II under growth conditions. According to the Fisher's LSD test, means followed by the same letter for each barley cultivar are not significantly different at $p < 0.05$. In the current study, the disease response of AS and AB infected with fungi without Si were reanalyzed for DI, DS; however, disease response of AS and AB infected with fungi was analyzed previously and cited by Sakr (2023a). A barley cultivar with lower value of DI (Type I resistance), DS (Type II) and AUDPC calculated on the basis of DI, Type I and DS, Type II was considered as more resistant than a barley cultivar with higher values DI (Type I resistance), DS (Type II) and AUDPC calculated on the basis of DI, Type I and DS, Type II

Table 2: Comparisons among reductions in disease incidence (DI) and disease severity (DS) and area under disease progress curve (AUDPC) calculated on the basis of DI and DS (% of control, inoculated with FHB pathogen and no addition of silicon) for a set of 16 fungal isolates for four Fusarium head blight species in barley (Arabi Aswad, AS and Arabi Abiad, AB) under controlled conditions

Fungal isolates (identification)	DI		DS		AUDPC calculated on the basis of DI		AUDPC calculated on the basis of DS	
	AS	AB	AS	AB	AS	AB	AS	AB
F1 (<i>F. culmorum</i>)	21a	17a	22a	18a	21a	16a	22a	18a
F2 (<i>F. culmorum</i>)	22a	17a	20a	18a	22a	17a	22a	18a
F3 (<i>F. culmorum</i>)	20a	18a	20a	19a	21a	16a	23a	19a
F28 (<i>F. culmorum</i>)	21a	19a	21a	19a	22a	17a	23a	19a
F30 (<i>F. culmorum</i>)	22a	17a	22a	20a	22a	16a	21a	20a
F7 (<i>F. solani</i>)	22a	18a	20a	18a	21a	17a	21a	20a
F31 (<i>F. solani</i>)	21a	18a	21a	18a	22a	18a	22a	20a
F35 (<i>F. solani</i>)	22a	17a	22a	19a	23a	18a	21a	18a
F20 (<i>F. solani</i>)	20a	18a	21a	19a	21a	16a	20a	19a
F26 (<i>F. solani</i>)	21a	17a	20a	20a	21a	17a	22a	19a
F29 (<i>F. solani</i>)	20a	19a	20a	20a	23a	18a	21a	19a
F15 (<i>F. verticillioides</i>)	21a	17a	20a	19a	23a	16a	21a	20a
F16 (<i>F. verticillioides</i>)	21a	18a	22a	19a	21a	17a	22a	20a
F21 (<i>F. verticillioides</i>)	20a	18a	22a	20a	21a	18a	21a	19a
F27 (<i>F. verticillioides</i>)	21a	19a	21a	20a	21a	17a	21a	19a
F43 (<i>F. equiesti</i>)	22a	18a	22a	19a	22a	16a	20a	18a

Values are means of three replicates. According to the Fisher's LSD test, values for the same cultivar treated with root Si irrigation among the 16 FHB isolates followed by the same letter in same column are not significantly different at $p < 0.05$.

DS was lower by 23 and 16%, respectively in experiment 1; 21 and 20%, respectively in experiment 2, and 22 and 17%, respectively in experiment 3 in moderately resistant and moderately susceptible +Si cultivars than barley plants amended with SDW (Figure 3, B).

Supply of Si concentration at 1.7 mM significantly reduced AUDPC calculated on the basis of DI on AS and AB by 21 and 17%, respectively in experiment 1; 21 and 14%, respectively in experiment 2, and 21 and 18%, respectively in experiment 3, as compared to these cultivars without Si (Figure 3, C).

AUDPC calculated on the basis of DS was reduced by Si treatments in AS and AB by 21 and 17%,

respectively in experiment 1; 24 and 19%, respectively in experiment 2, and 20 and 19%, respectively in experiment 3 than barley plants treated with SDW (Figure 3, D).

To check whether Si feeding strengthens the defense system measured by Type I and Type II on AB to a level comparable to AS not amended with Si, single degree of freedom contrasts test was used to compare the suppressive ability of the FHB damage expressed by DI, DS and AUDPC calculated on the basis of DI and DS between both cultivars: -Si AS and + Si AB. In all experiments, Si feeding in AB resulted in decreasing the head blight damage assessed by DI and DS to the same statistical level as that for the cultivar AS which is

moderately resistant without Si feeding.

Comparison among responses of four FHB species to root silicon irrigation

Values (% of control) of reductions of DI, DS as well as AUDPC calculated on the basis of DI and DS of four FHB species treated with treatment of exogenous Si irrigating at 1.7 mM through the root system, respectively on barley plants are shown in Table 2. Contrast analysis indicated that DI, DS and AUDPC calculated on the basis of DI and DS reductions were not significant among pathogen isolates with diverse pathogenicity ranging from highly to less pathogenic levels.

Si enhanced barley resistance under several experimental conditions

When Si applied to *Fusarium*-inoculated treatments under field conditions, all indicators of the Si-enhanced barley resistance were enhanced relative to fungal-inoculated treatments; Type I, Type II, Type I^{AUDPC}, and Type II^{AUDPC} were enhanced by 19.3%, 19.8%, 18.7% and 20.0%, respectively. Thus, these values of enhancement in resistance between -Si and +Si plants are comparable with those obtained under several experimental conditions, barley resistance components to FHB disease under *in vitro* and growth chamber conditions measured by LP, AUDPC, CLR, Type I, Type II, and Type III were enhanced by 17.7%, 17.5%, 17.7%, 18.7%, 20.3% and 20.2%, respectively.

DISCUSSION

Since *Fusarium* pathogens result in considerable damage to barley production across the world (Ogrodowicz *et al.*, 2020; Fernando *et al.*, 2021), several adopted control approaches has lost efficiency over time because of sudden weather changes and mutation in the pathogens that erode quantitative resistance potential in barley (Dahl and Wilson 2018; Janssen *et al.*, 2018). In this situation, Si has raised as a mineral element that decrease intensity of some fungal diseases in barley, such as powdery mildew and spot blotch (Wiese *et al.*, 2005; Holz *et al.*, 2022). In a previous growth chamber study (Sakr, 2021b); the combination of genetic resistance with Si amendment/foliar spraying on barley plants reduced the severity of highly aggressive

Fusarium isolates. In this study, we expanded the analysis of the effect of Si irrigation to the system root on barley resistance in two cultivars contrasting in terms of FHB resistance and infected with FHB isolates with diverse pathogenicity varying from highly to less aggressive levels. Evidence elucidates that higher levels of Si supplied by soil Si irrigating and absorbed by AS and AB leads to a decline in the intensity of disease symptoms associated with head blight. This decrease in FHB severity was not affected by the pathogenicity of the disease isolate but linked with the resistance levels of the host plants. These results showed that exogenous root irrigating application of Si provided barley resistance to *Fusarium* fungi.

Taking into account that the absorption of traditional Si generally takes place via plant roots as silicic acid (Guo-Chao *et al.*, 2018), we then used irrigating approach to make the root application with the Si concentration of 1.7 mM, which is the maximal solubility of Si in water and leads to optimum disease decrease in various pathosystems (Kaur and Greger, 2019). In field researches to define the effectiveness of Si for the management of plant diseases, Si crates mostly do not exceed 1.67 mM (Sakr, 2016; Debona *et al.*, 2017). Two transporters, *HvLsi1* and *HvLsi2*, responsible for the high ability for Si absorption in roots and shoots of barley have been identified by Chiba *et al.*, (2009) and Mitani *et al.*, (2009). Since the leaf blade in barley includes the highest Si rates (Deshmukh *et al.*, 2016), it can be hypothesized that the existence of soluble Si in the cytoplasm in barley shoots after its transporter (Chiba *et al.*, 2009; Mitani *et al.*, 2009) can increase resistance to *Fusarium* development in head tissues as observed previously in wheat-FHB association (Sakr, 2022b; Sakr and Kurdali, 2023) and other pathosystems, i.e., *B. graminis* f.sp. *tritici* in wheat (Guevel *et al.*, 2007) and *M. oryzae* in rice (Du *et al.*, 2022). Our results showed that Si absorption in barley root reinforced the defense system in head tissues to *Fusarium* invasion. FHB pathogens developed more severely on AS and AB plants grown without Si irrigation in which the barley's defense mechanisms are ineffective against *Fusarium* pathogens (Sakr, 2022c) in comparing to plants supplied with Si, showing that Si concentration increased only in

barley plants fed Si through the roots. Root application via Si irrigating may enhance development of barley root and results in efficient ability to control Si uptake to defeat FHB infection in the head tissues, which might play crucial function in coping with the tested *Fusarium* pathogens in the current investigation and previous reports on wheat (Sakr, 2022b; Sakr and Kurdali, 2023b). Our findings are in accordance with those reported by Du *et al.*, (2022) for the host cultivars infected with *M. oryzaei*, the causative agent of rice blast. Considering that roots play dynamic role in plant life cycle since they not only supply anchorage for plants developing aboveground, regulate the absorption of water and substantial nutrients from the soil, but also act as preservation of resources (Kaur and Greger, 2019), we conclude that this kind of promotion of root development under the irrigation system would aid with its absorption of Si to deal with FHB invasion.

The focus of any control policy which was utilized against plant diseases should fundamentally depend on the decrease of disease severity/incidence. Keeping this in mind, the four components, DI, DS and AUDPC calculated on the basis of DI and DS, evaluated in this study were negatively influenced by Si, highlighting that Si worked by reinforcing barley's defenses. The decrease of FHB pathogenic components in the growth chamber obtained by Si may be a consequence of the longer latent period, lower coleoptile length and diseased plant tissues as evidenced by the findings under *in vitro* trials (Sakr, 2023b). This outcome indicates that AS and AB plants supplied with Si responded more quickly to the pathogen's attack in which *Fusarium* fungi can penetrate the rachis and spread via direct floret-floret contamination in barley (Janssen *et al.*, 2018). The smallest pathogenic scores generated under diverse experimental conditions show that the progress of the disease in the head tissues of AS and AB was slower relative to fungal-inoculated-controls, which results in lower levels of diseased grains, which thus decreased the advance of head blight in the field, leading to lower disease intensity at the end of the barley cycle. A similar finding was also observed in +Si barley which defeated several destructive fungal pathogens, like *B. graminis* f. sp. *hordei* and *B.*

sorokiniana (Wiese *et al.*, 2005; Holz *et al.*, 2022), via alteration of their monocyclic criteria such as incubation duration, infection efficacy, lesion size, lesion expansion rate, and number of lesions per unit leaf area (Wiese *et al.*, 2005; Holz *et al.*, 2022).

While Si had no clear toxicity on FHB pathogens (Sakr, 2021a), we thus analyzed how Si irrigating in the root system reinforced the resistance of AS and AB to FHB disease invasion in the head tissues by stimulating barley defense reaction. In a more new report on FHB pathogen infecting wheat, Pazdiora *et al.*, (2023) described the biochemical nature of the Si-enhanced host resistance. The oxidation of phenolic compounds via polyphenol oxidase, the greater activity of antioxidant enzymes and the accumulation of soluble phenolic compounds in favor of the accumulation of hydrogen peroxide (H₂O₂), and lignification of tissues may have participated to the decrease in *Fusarium* colonization, leading to lower head blight intensity, particularly in wheat plants treated with Si. It is also hypothesized that these defense mechanisms participated to decrease the speed of *Fusarium* colonization in wheat treated with Si (Pazdiora *et al.*, 2023). Theoretically, our data hypothesis that the existence of soluble Si altered antioxidant enzyme and induced the release of higher soluble phenolic content that favored early accumulation of H₂O₂ and thereby resist the head invasion and enhance Type I resistance. In general, *H. vulgare* shows high Type II resistance, therefore the focus of breeding programs has been on enhancing Type I resistance. Improved Type I in +Si barley cultivars can synergistically prevent the development of the four tested *Fusarium* pathogens through enhancing cell wall deposits, forming papillae, and reinforcing the biosynthesis of hydrolase, thionine, lignin, and hydroxyproline-rich glycoprotein (Janssen *et al.*, 2018). Our data shed light on the complex interactions among *Fusarium* pathogens, host and Si and could result in enhanced management policies in FHB-barley pathosystem.

Pathogenicity of *Fusarium* is possibly the outcome of timely expression of several genes, governing release of cell-wall-degrading enzymes, mycotoxins, specific metabolites, and hormones that modify the host's

resistance response (Fernando *et al.*, 2021). It is hypothesized that Si would play a greater function in resistance against the more pathogenic isolate because of mostly weak induced defenses against the isolate (Vu *et al.*, 2022). In moderately susceptible and moderately resistant barley cultivars, Si decreased equally damage from the highest and least pathogenic isolates of the four tested *Fusarium* species regardless of pathogenic background for the used fungal materials. In contrast to our observations, Si frequently delayed lesion progress where rice plants were inoculated with the virulent isolate of bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*, but the impacts were less obvious where plants were inoculated with the less virulent isolate (Vu *et al.*, 2022). Furthermore, the Si-impact seemed to be virus-specific, since Si decreased *Tobacco ring spot virus* symptoms formation and did not alter *Tobacco mosaic virus* damage in tobacco plants (Zellner *et al.*, 2011). Such pathogen selectivity has also exhibited for certain fungal invasions (Rodgers-Gray and Shaw, 2004). In spite of the Si influence appears to be isolate/species-specific, the use of Si has been promoted as a more hopeful approach for the better control of plant diseases.

The best management of the disease under controlled climatic conditions in our investigation, indicated by the lower DI, DS and AUDPC calculated on the basis of DI and DS of the disease, was obtained with the use of Si in the soil under irrigation system, especially in AS. The level of FHB control was cultivar dependent as reported previously (Pazdiora *et al.*, 2023), leading to greater relative control in AS, which showed moderately resistance to head blight. This observation suggests that in AS, Si results in slower development of the FHB pathogens, and might be due to Si feeding interacts with the complex resistance mechanisms expressed by differential reactions conferred by quantitative trait loci mediated during FHB invasion, governing more resistance in AS. However, in this study, we showed that Si decreased head blight development in AB which shows a moderately susceptibility to head blight. This suggests that improved head blight resistance by Si is not limited to moderately resistant cultivar, AS. Thus, the decrease of FHB

severity was linked with the Si treatment and barley cultivar. Si uptake in barley improved the defense system on AB to a level comparable to AS not treated with Si, highlighting that Si uptake by the roots is required to avoid negative influence of *Fusarium* invasion. In accordance with our findings, the same reactions occurred on wheat challenged with the same tested FHB isolates under several experimental conditions (Sakr, 2022b; Sakr and Kurdali, 2023b). Earlier reports have proposed that soil Si could decrease the intensity of blast in susceptible rice cultivars to levels that are comparable with resistant cultivars (Rodrigues *et al.*, 2001). However, Xiao *et al.*, (2022) reported that although Si treatment decreased the powdery mildew intensity in strawberry, susceptible cultivar fed with Si had a disease intensity value four times that of resistant cultivar.

Importantly, Si application at 1.7 mM reduced FHB damage in previous analyses conducted on AS and AB under *in vitro* and field environments (Sakr, 2023a), suggesting that Si equally improved the expression of resistance to head blight infection in seedlings and adult barley plants. It seems that Si regulates multiple similarly signaling pathways involved in head and seedlings (Cho *et al.*, 2012) reaction to *Fusarium* infection. Augmenting evidence also demonstrated that Si plays a crucial role in several key components in plant signaling systems (Guo-Chao *et al.*, 2018; Kaur and Greger, 2019). In contrast to our data, no influence of soil Si on blast disease intensity in field plots at 34 days but observed a significant linear decline in disease intensity with increasing Si when the plots were re-sampled at 74 days (Klotzbucher *et al.*, 2018). Vu *et al.*, (2022) showed a clear plant age impact on the intensity of bacterial blight: disease lesions assessed at 32 days were largely uninfluenced by soil Si, but at 59 days, the impacts became increasingly obvious. The more apparent impacts in older, field-grown plants are possibly due to a greater accumulation of Si in plant tissues as they age (Klotzbucher *et al.*, 2018; Vu *et al.*, 2022).

CONCLUSIONS

Research on the treatment of Si for barley disease inhibition is in its infancy; however, Si may provide an

additional component for the control of FHB in barley, as it reinforces plant defense responses. The greatest advantage will be obtained when used with AS cultivar with moderately resistant. In the case of moderately susceptible cultivar, AB; Si reduced all features of FHB pathogenicity on AB to the same levels as observed on AS. Root treatment with Si might enhance the root growth of barley resulting in its enhanced ability of Si uptake and a better reaction to FHB disease. More physiological, cytological and biochemical analyses would be required to explore how Si can promote barley defenses to FHB invasion. All of these findings are promising outcomes for the application of Si as an effective and safe policy against FHB damage.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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