



Silicon soil amendment as a complement to manage tan spot and fusarium head blight in wheat

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Abstract

Tan spot, caused by *Pyrenophora tritici-repentis*, and fusarium head blight (FHB), caused by the *Fusarium graminearum* species complex, are among the main wheat diseases worldwide. This 3-year field study evaluated the effect of soil incorporation of calcium silicate, a source of silicon (Si), to manage tan spot and FHB and improve grain yield and quality. The effect of Si was compared on two cultivars contrasting in disease resistance and associated with one or two fungicide sprays. Calcium silicate fertilization increased the Si concentration in the soil and wheat leaf and spike tissues. The increase of Si concentration in wheat tissues was associated with reduction in the severity of both tan spot and FHB, consequently increased the quality and grain yield of wheat. The reduction of disease severity conferred by Si was greater for tan spot than FHB. The greatest control of tan spot and FHB was obtained with the moderately resistant cultivar grown in soil amended with calcium silicate (+Si) and treated with two fungicide sprayings. On the other hand, the highest grain yield, under high disease pressure, was obtained in +Si plants, regardless of the cultivar, treated with two fungicide sprays. The results of this study show for the first time that the incorporation of Si in the soil complemented the effect of genetic resistance and fungicide treatments in controlling both tan spot and FHB. Furthermore, results indicate that calcium silicate fertilization is useful as part of integrated management of these wheat diseases.

Keywords *Pyrenophora tritici-repentis* · *Fusarium graminearum* · Calcium silicate · Disease control · Integrated disease management · Wheat disease

1 Introduction

Wheat (*Triticum aestivum* L.) is the most consumed and second most produced cereal worldwide, and is a staple food for more than 36% of world's population (FAO 2020; Li et al. 2015). Tan spot (Fig. 1a), caused by *Pyrenophora tritici-repentis*, and fusarium head blight (FHB) (Fig. 1b), caused by the *Fusarium graminearum* species complex, are two of the main wheat diseases worldwide. Tan spot affects the leaves and spikes, causing a reduction in grain yield by up to 48%, mainly due to the reduction in the number and weight of grains per spike (Rees and Platz 1983; Ronis et al. 2009).

The FHB pathogen reduces grain yield by up to 50% and also contaminates grain with mycotoxins, especially trichothecenes such as deoxynivalenol, which poses a serious threat to food safety (Bai and Shaner 1994; McMullen et al. 2012).

The management of these diseases can be accomplished by sowing healthy seeds, and using crop rotation and tillage to reduce inoculum (Bockus and Claassen 1992; Carmona et al. 2006). Fungicides are also used to control tan spot and FHB, but chemical treatment adds cost to wheat growers, and sometimes, for FHB, the results of their application are not satisfactory (D'Angelo et al. 2014). Planting of resistant cultivars is considered an effective and environmentally friendly approach to reduce damage caused by foliar and spike diseases (Halder et al. 2019). Unfortunately, resistance to FHB is largely quantitatively inherited and limited by additive genetic effect and genotype × environment interaction, making it available only as moderately resistant cultivars (Buerstmayr et al. 2020; Halder et al. 2019). Moderate resistance of cultivars to tan spot and FHB is associated with higher plant defense through cell wall fortification, and increased biochemical

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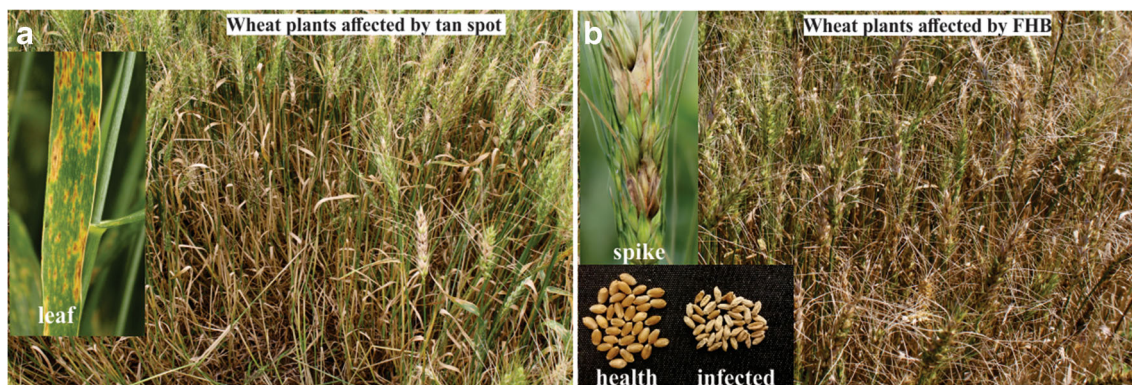


Fig. 1 Wheat diseases. **(a)** Wheat plants, affected by tan spot, showing all leaves killed by the pathogen *Pyrenophora tritici-repentis*. Details of tan spot symptoms on wheat leaf (left image). **(b)** Spikes of wheat showing symptoms of fusarium head blight (FHB) (right image). Spike partially

affected by FHB with spikelets exhibiting premature bleaching and aggregations of light pink/salmon colored spores (right; upper); and comparison of grains produced on health and infected spikes (right; bottom)

defense mechanisms that restrict pathogen colonization and/or mycotoxin accumulation (Pritsch et al. 2000; Strelkov and Lamari 2003; Walter et al. 2010; Ding et al. 2011; Dorneles et al. 2017).

With respect to wheat defense against tan spot and FHB, silicon (Si) is an attractive complement to enhance the defense mechanisms of the plants, especially as a factor for integrated control of the diseases. In wheat, several fungal diseases (blast - *Magnaporthe oryzae* Triticum pathotype; tan spot, septoria leaf blotch - *Zymoseptoria tritici*; spot blotch - *Cochliobolus sativus*; and powdery mildew - *Blumeria graminis* f. sp. *tritici*) were found to be reduced by Si through alteration of their monocyclic components such as incubation period, infection efficiency, lesion expansion rate, lesion size, and number of lesions per unit leaf area (Rodrigues et al. 2015; Dorneles et al. 2017; Pazdiora et al. 2018). The effect of Si on the reduction of disease intensity is basically attributed to two mechanisms that act additively and/or synergistically: physical barrier and biochemical defenses (Debona et al. 2017). In wheat, the physical barrier was reported to be due to Si deposition below the cuticle and the increase of papillae deposition at infection sites (Bélanger et al. 2003; Xavier-filha et al. 2011). Furthermore, Si primed the biochemical defenses by increasing defense enzyme activity, biosynthesis of phenolic compounds and phytoalexins, and accumulation of hydrogen peroxide at the infection sites (Rodrigues et al. 2015; Debona et al. 2017; Dorneles et al. 2017, 2018).

Taking into account the effect of Si against leaf diseases and wheat defense to tan spot and FHB, we believe that wheat plants supplied with Si may be less affected by both leaf and spike diseases, and therefore allowing a reduction in the number of fungicide sprays during the crop cycle. Thus, the objective of this study was to evaluate the potential of Si soil amendment to reduce disease and yield damage caused by tan spot and FHB. The Si effects were analyzed on two wheat cultivars contrasting in resistance to tan spot and FHB associated to fungicide treatment with one (at the stem elongation stage) and two (at stem elongation and flowering stages) sprays.

2 Material and methods

2.1 Study site, cultural practices, and treatments

Field experiments were conducted at the Palma Agriculture Center (31°48'06.4"S 52°30'18.6"W) belonging to the Federal University of Pelotas. The same experiment was conducted in three successive field seasons, 2016, 2017, and 2018. In each year, the experiment was established in a conventionally tilled field, after burying previous crop residues. The area used was a former orange orchard and the 2016 crop season was the first year of cultivation with wheat.

The physicochemical characteristics of the soil were as follows: 19% clay, 1.79% organic matter, Ca^{2+} , Mg^{2+} , Al^{3+} , $\text{H}+\text{Al}^{3+} = 3.2, 0.7, 0.2, 2.2 \text{ cmolc.kg}^{-1}$, respectively, cation exchange capacity (1.0 M $\text{CH}_3\text{CO}_2\text{NH}_4$) $4.2 \text{ cmolc.kg}^{-1}$, base saturation = 85%, pH (CaCl_2) = 4.3, P and K (Mehlich 1) = 7.2 and 36 mg.kg^{-1} , respectively. The concentration of available Si (extracted with 0.01M CaCl_2) was 6.2 mg.kg^{-1} . The soil fertility was adjusted, as described below, using chemical fertilizer to achieve a yield of 4.0 t.ha^{-1} .

Calcium silicate (Agrosilício Plus®, Agronelli Insumos Agrícolas, Uberaba, Brazil), composed of 10.5% Si, 25.0% Ca, and 6.0% Mg, was the source of Si. In the 2016 and 2017 crop seasons, the product was incorporated into the soil at a rate of 4.0 t.ha^{-1} in order to increase the soil pH to 6.5. To standardize the amount of calcium and magnesium supplied to the plants in the calcium silicate treatment, the soil in the control treatment was amended with extra-fine limestone at the rate of 3.33 t.ha^{-1} . The extra-fine limestone (Dagoberto Barcelos, Caçapava do Sul, Brazil) was composed of 26.5% Ca and 15.0% Mg. In the 2018 crop season, 2.0 t.ha^{-1} of calcium silicate or extra-fine limestone was added to the soil. For all seasons, calcium silicate or limestone was incorporated in the soil by harrowing 30 days before sowing.

Soil samples collected on the day of sowing (2016 crop season) indicated concentrations of 3.5 and $3.4 \text{ cmolc.kg}^{-1}$

of Ca^{2+} and 10.8 and 6.2 mg.kg^{-1} of Si, respectively, in the plots that received calcium silicate and extra-fine limestone (Fig. 2). In the second year of the experiment (2017 season), soil analysis indicated 5.1 and 4.3 cmolc.kg^{-1} of Ca^{2+} and 13.8 and 6.2 mg.kg^{-1} of Si, respectively, in the plots that received calcium silicate or extra-fine limestone. In 2018, soil analysis indicated 5.2 and 4.5 cmolc.kg^{-1} of Ca^{2+} and 15.4 and 6.2 mg.kg^{-1} of Si, respectively, in the plots that received calcium silicate and extra-fine limestone.

Two wheat (*Triticum aestivum*) cultivars were chosen – Tbio Toruk (Biotrigo®) and Tbio Sossego (Biotrigo®) – from among regionally adapted cultivars with similar anthesis and maturity dates, but contrasting in disease resistance, the former being more susceptible to tan spot and FHB than the latter. Plots were planted (9 rows, 0.17 m row spacing) with a plot drill (Semeato, SHP model) at density of 300 plants per m^2 . Chemical fertilizer (05-20-20 nitrogen, phosphorus, potassium) at rate of 300 kg.ha^{-1} was applied at sowing. Total nitrogen (granular urea, N, 45%) input was 100 $\text{kg N ha}^{-1} \text{yr}^{-1}$, where 15% of the total N was applied as basal fertilizer and the remainder (85%) was top-dressed at the phenological stages of tillering and stem elongation (growth stage (GS) 25 and GS37). Iodosulfuron-methyl (Hussar®; Bayer, 100 g c.p. ha^{-1}) and imidacloprid beta-cyfluthrin (Connect®; Bayer, 500 mL c.p. ha^{-1}) were used to control weeds and insects,

respectively, as necessary to ensure these factors would not influence the outcome of the experiment.

The fungicide prothioconazole (150 g L^{-1} ; triazolintione) + trifloxystrobin (175 g L^{-1} ; strobilurin) (Fox®; Bayer, 0.5 c.p. ha^{-1}) was applied at the stem elongation stage (GS32/GS33) (one spraying treatment) alone or at both the stem elongation stage and at flowering (GS60) (two spraying treatments). The fungicide was applied using a CO_2 pressured sprayer kit, with four tip nozzles (TTJ60 11002; Teejet®), delivering 200 L ha^{-1} .

2.2 Experimental design

The experimental design was a three-way ($2 \times 2 \times 2$) factorial scheme in a randomized block (two silicon treatments - not supplied (-Si) or supplied (+Si); two cultivars – Tbio Toruk or Tbio Sossego; and two fungicide treatments – one application or two applications), with four replications. Each experimental plot was 1.53 m \times 5 m, separated by 1 m and a 30 cm deep furrow to prevent runoff of rainwater from one plot to the other (Fig. 3).

2.3 Disease assessment

The disease assessment was conducted from tillering to early dough (GS83). Powdery mildew (*Blumeria graminis* f. sp.

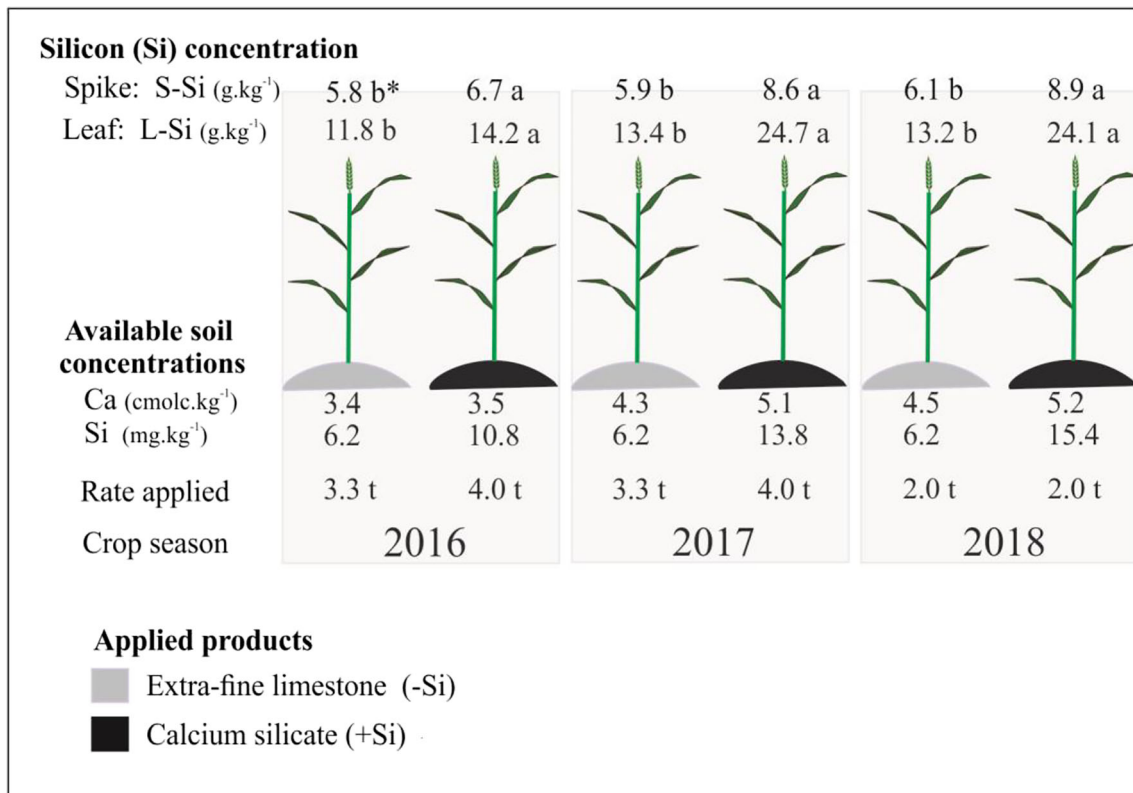
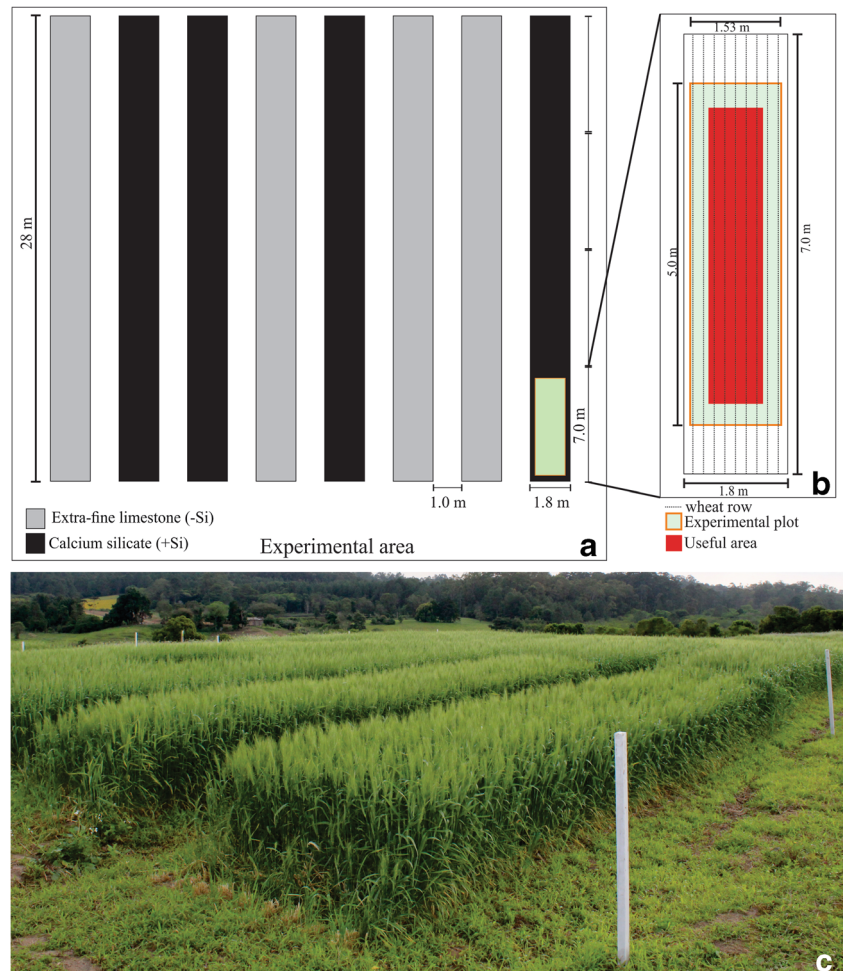


Fig. 2 Rate applied of extra-fine limestone (-Si) or calcium silicate (+Si), concentration of silicon (Si) and calcium (Ca) available in the soil at sowing and leaf (L-Si) and spike (S-Si) silicon concentration for each

crop season. *Means for silicon concentration in the leaves or spikes, for each season, followed by different letters are statistically significant different by Tukey test at $P \leq 0.05$

Fig. 3 Representation (not to scale) of the area used for the experiment and distribution of blocks that received extra-fine limestone (-Si) or calcium silicate (+Si) (a). Details of the plot showing the area considered to be experimental plot (yellow rectangle) with nine rows (b). All evaluations were conducted in the useful area of the plot (five central rows, each with length of 4 m). View of the experimental area showing blocks and plots (c)



tritici) and Septoria leaf blotch (*Zymoseptoria tritici*) were observed only occasionally on plants of some treatments and in some years, but with low incidence (less than 20%) and low severity (less than 1%). The main diseases were tan spot and FHB.

In 2017 and 2018, tan spot incidence was 100% regardless of the treatment since stem elongation, so only the severity was measured, according to Pazdiora et al. (2018), by quantifying the percentage of the leaf area showing disease symptoms relative to the total leaf area of ten plants randomly chosen at five different locations, resulting in total of 50 plants per plot. As in 2016 the tan spot occurred only after flowering stage, the data presented for the 3 years are the latest evaluation conducted at early dough.

The FHB incidence was measured at early dough stage by counting the number of spikes with typical FHB symptoms (i.e., fully or partially bleached spikes). For each experimental plot, 100 spikes from the central line were observed, resulting in 400 spikes per treatment. Disease incidence was expressed as the percentage of symptomatic wheat spikes. The FHB severity was the percentage of spikelets with typical FHB symptoms relative to the total number of spikelets per spike.

The FHB severity was measured in the same spikes used for FHB incidence evaluation.

2.4 Yield measurement

Wheat was hand harvested from 3.4 m² (five rows with four meters) and threshed in a mechanical grain thresher (EDA, model TR. PARCELA). The grain moisture content of a representative subsample was measured with a moisture tester (Gehaka Agri G600). Grain yield was measured with a balance and normalized to 13% grain moisture content. A sample of 1000 grains, counted by an electronic seed and grain counter (Sanick ESC 2011), was weighed on an analytical balance (Shimadzu model BL 3200H) and expressed in grams to determine the 1000 grain weight. The hectoliter weight (HW) was measured using a cereal weight scale (Dallemolle type 40) according to international standards.

2.5 Determination of Si concentration

Flag leaves and spikes were sampled at the flowering stage (GS60), rinsed with deionized water, dried for 72 h at 70 °C,

and ground using a mortar and pestle to pass through a 40-mesh screen. Foliar and spike Si concentrations were determined by colorimetric analysis of 0.1 g of alkali-digested tissue (Domeles et al. 2017).

2.6 Data analysis

Prior to Anova, Levene's test was used to assess whether homoscedasticity could be assumed. After applying the parametric ANOVA, the Shapiro–Wilk test was applied to ascertain normality of the fitted residuals. Data of the three years were analyzed separately (Tables 1 and 2) and means compared by Tukey test ($P \leq 0.05$). All the statistical analyses were performed using the computing environment R (R Core Team 2020), using “agricolae” (Mendiburu 2014) and “car” (Fox and Weisberg 2019) packages.

3 Results

Extra-fine limestone amendment in the soil increased the concentration of calcium (Ca) in the soil during the three growing seasons, but not the silicon (Si) concentration (Fig. 2). However, calcium silicate amendment increased the concentration of both Si and Ca in the soil over the three seasons (Fig. 2). The highest concentration of Si in the soil resulted in the significantly higher concentration of the element in the leaf and spike (Fig. 2).

The temperatures during the wheat cycle were favorable to diseases (tan spot and FHB) in the three seasons (Fig. 4). However, the rain distribution was more variable. The 2016 season was marked by drought during the stem elongation and booting stages, but precipitation was normal after the flowering stage (Fig. 4). In 2017 and 2018, rainfall was distributed more evenly across the wheat cycle.

Table 1 Analysis of variance for tan spot severity, fusarium head blight (FHB) incidence, and FHB severity on plants of the cultivars (cultivar) Tbio Toruk and Tbio Sossego grown in soil amended (soil corrective)

with calcium silicate or extra-fine limestone treated with fungicide (Fung) at the stem elongation stage or stem elongation plus flowering stages during the 2016, 2017, and 2018 growing seasons

Year, variation	Tan spot severity			FHB incidence			FHB severity		
	df	F value	P value	df	F value	P value	df	F value	P value
2016									
Cultivar (cv)	1	57.2410 **	<.0001	1	10.7664 **	0.0031	1	1.0548 ns	0.3145
Soil corrective (Sc)	1	57.2410 **	<.0001	1	0.3718 ns	0.5476	1	0.2763 ns	0.6038
Fungicide sprays (Fung)	1	551.9556 **	<.0001	1	7.6096 *	0.0109	1	5.6827 *	0.0254
cv × Sc	1	1.4271 ns	0.2438	1	0.1897 ns	0.6669	1	0.1804 ns	0.6746
cv × Fung	1	3.9641 ns	0.0578	1	1.1543 ns	0.2932	1	0.2086 ns	0.6518
Sc × Fung	1	3.9641 ns	0.0578	1	1.1543 ns	0.2932	1	0.0608 ns	0.8071
Cv × Sc × Fung	1	3.9641 ns	0.0578	1	0.9182 ns	0.3473	1	1.3333 ns	0.2594
2017									
Cultivar (cv)	1	19.6909 **	0.0001	1	7.8142 *	0.01	1	5.7473 *	0.0246
Soil corrective (Sc)	1	45.8727 **	<.0001	1	0.6669 ns	0.422	1	4.3493 *	0.0478
Fungicide sprays (Fung)	1	12.2727 **	0.0018	1	37.5123 **	<.0001	1	37.4021 **	<.0001
cv × Sc	1	0.4909 ns	0.4901	1	1.1404 ns	0.296	1	0.0205 ns	0.8871
cv × Fung	1	0.0545 ns	0.8172	1	0.0483 ns	0.8277	1	0.6242 ns	0.4371
Sc × Fung	1	1.3636 ns	0.2542	1	0.1934 ns	0.6639	1	0.1375 ns	0.7138
Cv × Sc × Fung	1	0.0545 ns	0.8172	1	0.0631 ns	0.8036	1	0.3629 ns	0.5524
2018									
Cultivar (cv)	1	227.8763 **	<.0001	1	4.2640 *	0.0464	1	6.5455 *	0.0172
Soil corrective (Sc)	1	219.3760 **	<.0001	1	16.5838 **	0.0004	1	58.9091 **	<.0001
Fungicide sprays (Fung)	1	30.1955 **	<.0001	1	5.4975 *	0.0276	1	4.5455 *	0.0434
cv × Sc	1	94.6117 **	<.0001	1	1.8426 ns	0.1871	1	4.5455 *	0.0434
cv × Fung	1	12.7350 **	0.0015	1	1.8426 ns	0.1871	1	0.7273 ns	0.402
Sc × Fung	1	0.0040 ns	0.95	1	1.2335 ns	0.2776	1	0.7273 ns	0.402
Cv × Sc × Fung	1	0.6233 ns	0.4374	1	0.0152 ns	0.9027	1	0.1818 ns	0.6735

** significant at $P < 0.01$; * significant at $P < 0.05$; ns – not significant

Table 2 Analysis of variance for grain yield, weight of 1000 grains, and hectoliter weight of plants of the cultivars (cultivar) Tbio Toruk and Tbio Sossego grown in soil amended (soil corrective) with calcium silicate or extra-fine limestone treated with fungicide (Fung) at the stem elongation stage or stem elongation plus flowering stages during the 2016, 2017, and 2018 growing seasons

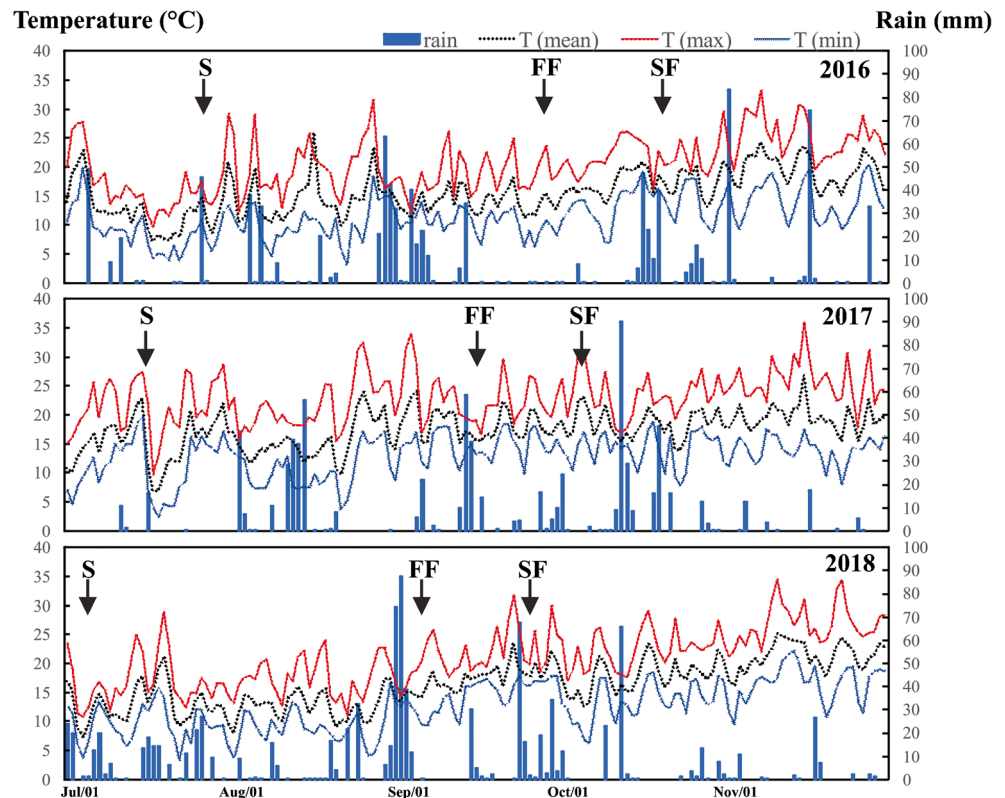
Year, variation	Yield			1000 grain weight			hectoliter weight		
	df	F value	P value	df	F value	P value	df	F value	P value
2016									
Cultivar (cv)	1	13.1717 **	0.0013	1	70.4052 **	<.0001	1	0.1144 ns	0.7379
Soil corrective (Sc)	1	1.3098 ns	0.2636	1	0.9161 ns	0.3479	1	0.5793 ns	0.4539
Fungicide sprays (Fung)	1	0.7750 ns	0.3873	1	0.1808 ns	0.6743	1	51.6687 **	<.0001
cv × Sc	1	0.0187 ns	0.8922	1	3.0624 ns	0.0927	1	14.4815 **	0.0008
cv × Fung	1	0.0263 ns	0.8724	1	0.0654 ns	0.8001	1	0.1788 ns	0.676
Sc × Fung	1	0.1700 ns	0.6836	1	2.8299 ns	0.1053	1	0.4577 ns	0.505
Cv × Sc × Fung	1	0.4950 ns	0.4883	1	1.2388 ns	0.2766	1	0.7151 ns	0.4059
2017									
Cultivar (cv)	1	10.8617 **	0.003	1	10.3471 **	0.0041	1	3.6698 ns	0.0689
Soil corrective (Sc)	1	7.7383 *	0.0103	1	7.0623 *	0.0147	1	4.3489 *	0.0494
Fungicide sprays (Fung)	1	36.1455 **	<.0001	1	35.9326 **	<.0001	1	42.2283 **	<.0001
cv × Sc	1	0.3335 ns	0.5688	1	1.2684 ns	0.2725	1	0.0971 ns	0.7583
cv × Fung	1	6.5919 *	0.0169	1	9.9971 **	0.0047	1	9.4996 **	0.0056
Sc × Fung	1	4.4528 *	0.0454	1	4.8409 *	0.0391	1	1.2738 ns	0.2716
Cv × Sc × Fung	1	0.6170 ns	0.4397	1	0.5430 ns	0.4692	1	0.0545 ns	0.8176
2018									
Cultivar (cv)	1	2.9455 ns	0.0988	1	1.0440 ns	0.3169	1	15.0227 **	0.0081
Soil corrective (Sc)	1	14.6157 **	0.0008	1	9.1864 **	0.0057	1	4.7186 *	0.0456
Fungicide sprays (Fung)	1	3.1210 ns	0.0899	1	15.4819 **	0.0005	1	17.0978 **	0.0003
cv × Sc	1	0.0027 ns	0.9588	1	4.4808 *	0.0448	1	2.4605 ns	0.1297
cv × Fung	1	1.0487 ns	0.3159	1	2.9384 ns	0.0992	1	0.2904 ns	0.5948
Sc × Fung	1	1.0752 ns	0.31	1	2.1536 ns	0.1551	1	0.3557 ns	0.5563
Cv × Sc × Fung	1	0.0000 ns	0.9973	1	10.1998 **	0.0038	1	0.1002 ns	0.7541

** significant at $P < 0.01$; * significant at $P < 0.05$; ns – not significant

In 2017 (Fig. 5b) and 2018 (Fig. 5c), the meteorological conditions were more conducive to tan spot than 2016, when the disease severity was lower than 10% (Fig. 5a). The effects of the higher resistance in the cultivar Tbio Sossego were pronounced in 2017 (Fig. 5b) and 2018 (Fig. 5c), significantly reducing the tan spot severity compared to the Tbio Toruk cultivar, regardless of the fungicide and soil amendment treatments. The amendment with calcium silicate (+Si) in the soil significantly reduced tan spot severity, especially in 2017 (Fig. 5b) and 2018 (Fig. 5c), regardless of the cultivar and fungicide treatment. We would point out that, generally, tan spot severity was lower in +Si plants treated with fungicide at the stem elongation stage than –Si plants treated twice with fungicide (spraying at stem elongation and flowering stages). When comparing the same cultivar with the same soil amendment, two fungicide sprayings were always more effective than one at the stem elongation stage in reducing tan spot severity (Fig. 5a–c).

The effect of treatments (cultivars, soil amendments, and fungicide application) on tan spot severity can be visualized in Fig. 6. The higher resistance to tan spot of the cultivar Tbio Sossego (Fig. 6i) relative to Tbio Toruk (Fig. 6a) is clearly evident by comparing the –Si plants treated with fungicide applied at the stem elongation stage. The leaves of the plants of Tbio Toruk were affected by the disease up to the flag leaf, while up to two green leaves were still visible in the Tbio Sossego plants. The effect of calcium silicate was clearly evident in plants treated with a single fungicide application at the stem elongation stage for both cultivars (Fig. 6b and j). For Tbio Toruk, +Si plants (Fig. 6b) retained at least two green leaves while in –Si plants (Fig. 6a), the disease affected all leaves. In the cultivar Tbio Sossego, a higher number of leaves remained green in +Si plants (Fig. 6j) than in –Si plants (Fig. 6i). For both cultivars, calcium silicate amendment in the soil plus one spraying of fungicide at the stem elongation stage (Fig. 6b and j) conferred a similar number of green leaves to

Fig. 4 Rainfall, mean (Tmean), maximum (Tmax), and minimum (Tmin) temperature during the growing cycle of wheat in the three seasons (2016, 2017, and 2018). S = date of sowing; FF = date of the first fungicide spraying (at stem elongation stage); SF = date of the second fungicide spraying (at flowering)



plants that received two sprayings in soil amended with extra-fine limestone ($-Si$) (Fig. 6c and k). Plants of both cultivars that received two fungicide sprayings showed higher proportion of green leaf area than plants with one spraying, and the tan spot control was the highest for $+Si$ plants receiving two sprayings (Fig. 6d and l).

For FHB, the effect of the higher genetic resistance present in the cultivar Tbio Sossego was observed in 2016, 2017, and 2018 for FHB incidence (Fig. 5d–f) and in two crop seasons (2017 and 2018) for FHB severity (Fig. 5g and h). The lower FHB incidence and severity in spikes of the cultivar Tbio Sossego (Fig. 6m and n) compared to Tbio Toruk (Fig. 6e and f) was also clearly observed in the field, especially for plants without fungicide application at flowering. Calcium silicate reduced the FHB incidence only in Tbio Sossego plants treated with fungicide at flowering (2016 and 2017) (Fig. 5d and e) and for both fungicide treatments in 2018 (Fig. 5f). However, lower FHB severity was recorded in $+Si$ plants than $-Si$ ones, of both cultivars, regardless of the number of fungicide applications, in 2017 (Fig. 6h) and 2018 (Fig. 6p), except for Tbio Toruk treated with fungicide at the stem elongation stage in 2017. In general, fungicide spraying at the flowering stage significantly reduced the FHB incidence, especially in 2017 and 2018, regardless of the cultivar or soil amendment, and for the $+Si$ plants of Tbio Sossego in 2016. On the other hand, the FHB severity was reduced

significantly by fungicide treatment at flowering regardless of the cultivar or soil corrective in the three crop seasons.

The grain yield of Tbio Toruk was higher than that of Tbio Sossego, regardless of fungicide and soil amendment, in 2016 (Fig. 7a); in plants receiving two fungicide sprayings, in 2017 (Fig. 7b); and for $+Si$ plants treated with fungicide at the stem elongation stage in 2018 (Fig. 7c). The cultivar Tbio toruk also showed higher grain weight regardless of the fungicide and soil amendment in 2016 (Fig. 7d), and for $+Si$ plants in 2017 (Fig. 7e) and 2018 (Fig. 7f). Calcium silicate, compared to extra-fine limestone, significantly increased the grain yield in 2017 (Fig. 7b) and 2018 (Fig. 7c), except for plants that received two fungicide sprayings in 2017. Grain weight (Fig. 7e) and hectoliter weight (Fig. 7h) were only increased by calcium silicate in plants treated with fungicide at the stem elongation stage in 2017. In 2018, calcium silicate significantly increased the grain weight of Tbio Toruk with two fungicide sprayings (Fig. 7f), and the hectoliter weight of grains of Tbio Toruk (Fig. 7i), regardless of the number of fungicide sprays. In general, the fungicide treatment at flowering increased the grain yield and the weight of grains, especially in 2017. Furthermore, hectoliter weight also increased due to fungicide application at flowering, regardless of the cultivar or soil amendment, in 2017 (Fig. 7h) and 2018 (Fig. 7i).

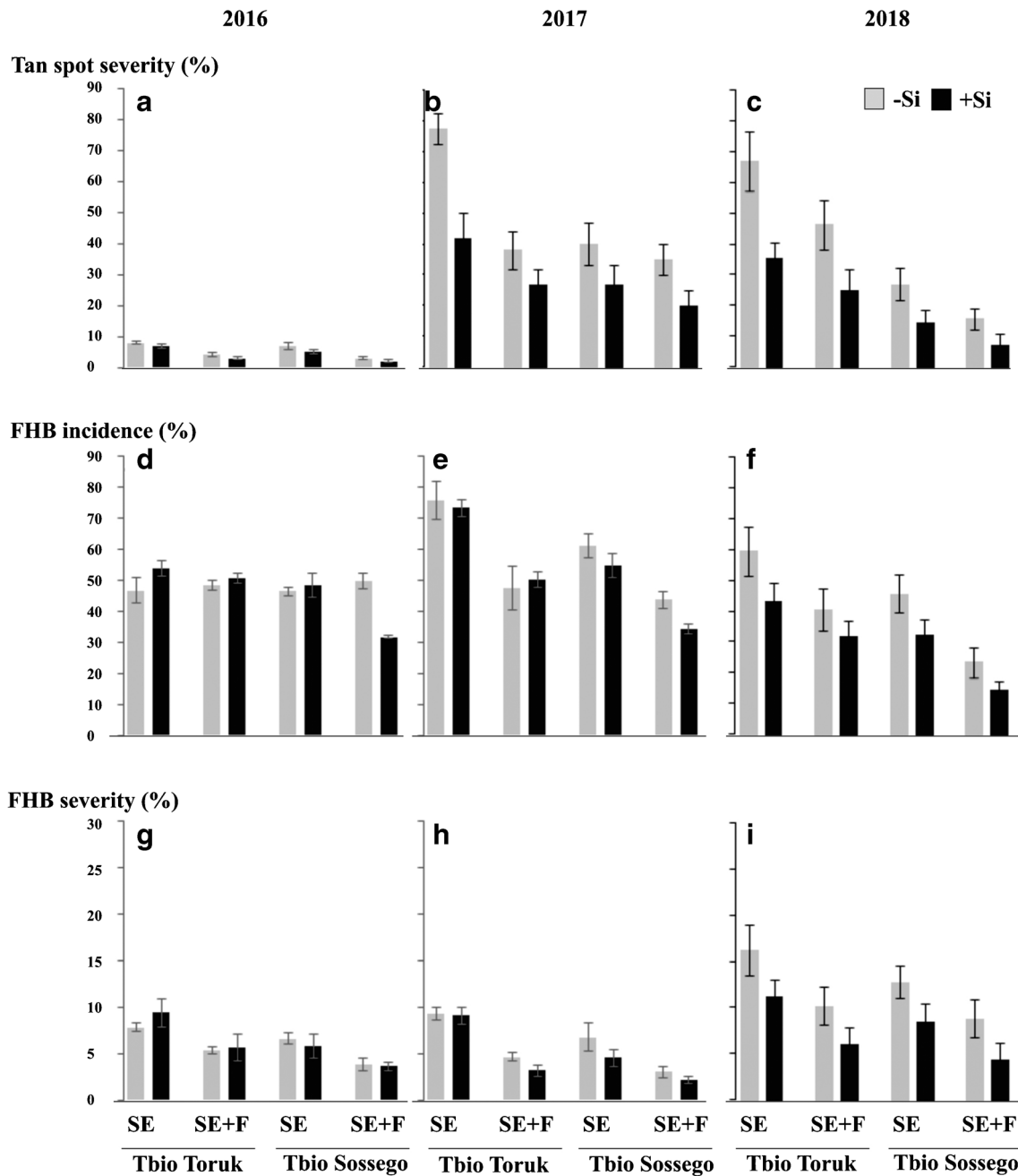


Fig. 5 Tan spot severity (a–c) on the leaves, fusarium head blight (FHB) incidence (d–f), and FHB severity (g–i) on the spikes of wheat plants of the cultivars Tblio Toruk and Tblio Sossego grown in soil amended with extra-fine limestone (–Si) or calcium silicate (+Si) and treated with

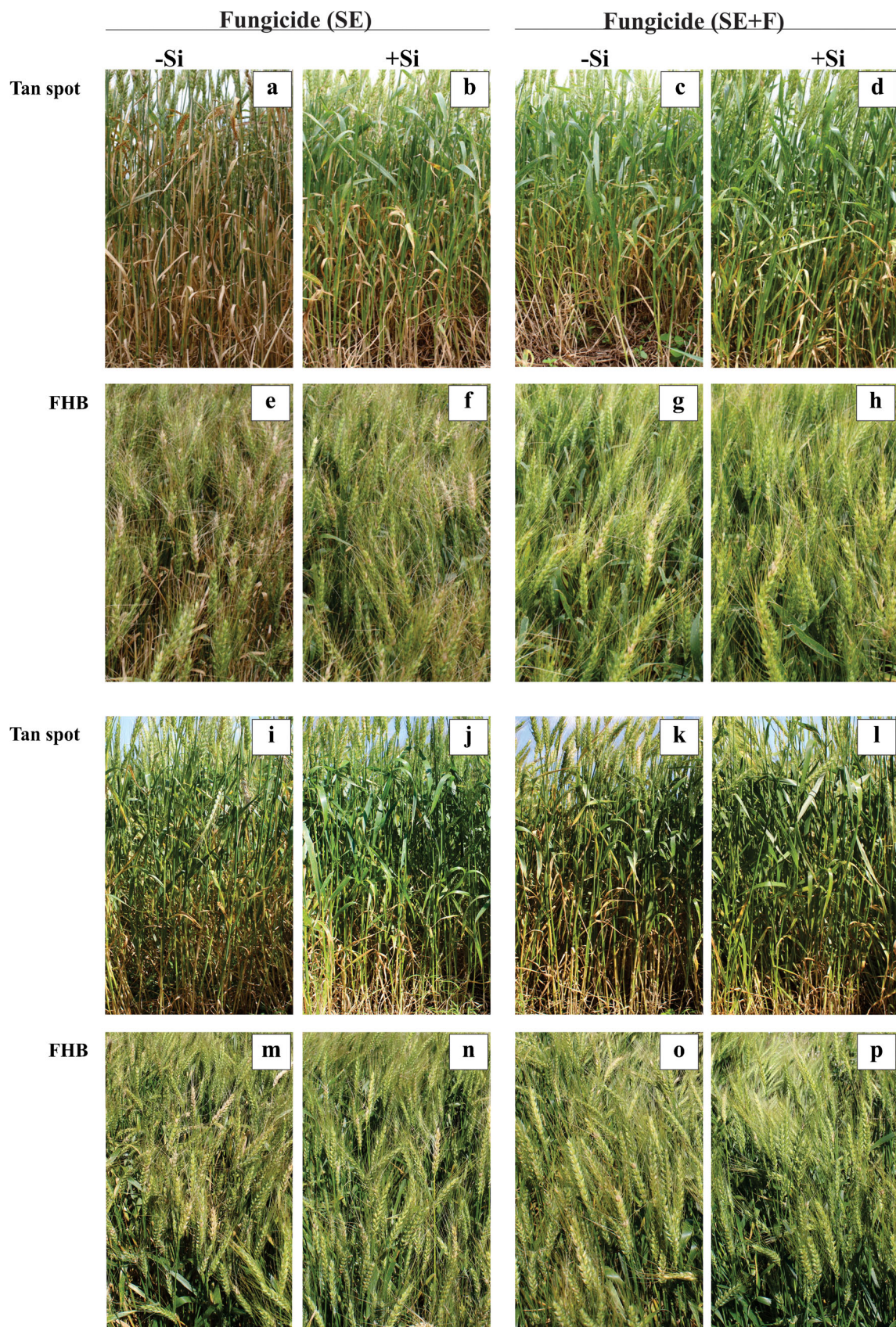
fungicide at the stem elongation stage (SE) or at stem elongation and flowering stages (SE+F) in 2016 (a, d, and g), 2017 (b, e, and h) and 2018 (c, f, and i). Error bars represent standard deviation of means

4 Discussion

In previous studies, under greenhouse environment, calcium silicate treatment of wheat has been shown to reduce tan spot severity by altering several epidemic components (Pazdiora et al. 2018). This effect caused by calcium silicate fertilization occurred due to increasing the silicon (Si) concentration in wheat leaves, which has been associated with earlier and stronger activation of the plant's capacity to defend itself against

fungicide at the stem elongation stage (SE) or at stem elongation and flowering stages (SE+F) in 2016 (a, d, and g), 2017 (b, e, and h) and 2018 (c, f, and i). Error bars represent standard deviation of means

Fig. 6 Leaf area affected by tan spot (a–d and i–l) and spikes affected by fusarium head blight (FHB) (e–h and m–p) of wheat plants of the cultivars Tblio Toruk (a–h) and Tblio Sossego (i–p) grown in soil amended with extra-fine limestone (–Si) (a, c, e, g, i, k, m, and o) or calcium silicate (+Si) (b, d, f, h, j, l, n, and p) and treated with fungicide at the stem elongation stage (SE) (a, b, e, f, i, j, m, and n) or at stem elongation and flowering stages (SE+F) (c, d, g, h, k, l, o, and p)



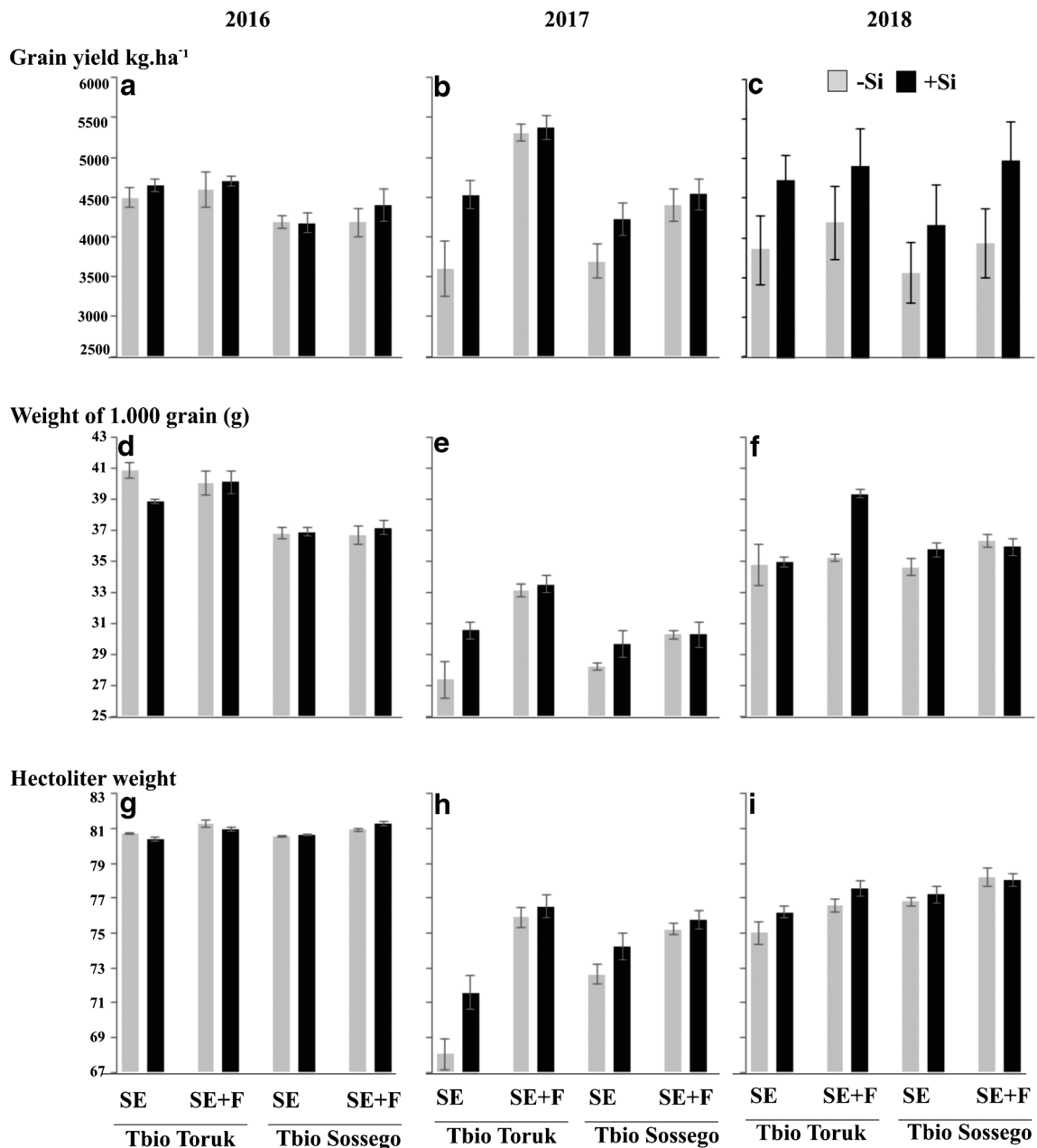


Fig. 7 Grain yield (a–c), weight of 1000 grains (grain weight) (d–f) and hectoliter weight (g–i) of grains produced by wheat plants of the cultivars Tblio Toruk and Tblio Sossego grown in soil amended with extra-fine limestone (–Si) or calcium silicate (+Si) and treated with fungicide at

the stem elongation stage (SE) or at stem elongation and flowering stages (SE+F) in 2016 (a, d, and g), 2017 (b, e, and h) and 2018 (c, f, and i). Error bars represent standard deviation of means

P. tritici-repentis infection through several defense mechanisms, including superoxide dismutase, peroxidase, chitinase, and phenylalanine ammonia-lyase activities, as well as the accumulation of hydrogen peroxide and derivatives of the phenylpropanoid pathway (Dorneles et al. 2017, 2018). The current study shows for the first time the increase in the Si concentration in the leaves and spikes in wheat plants grown in field conditions in soil amended with calcium silicate, which reduced tan spot and FHB intensities, consequently increasing grain yield. Furthermore, this study also reports for the first

time the integration of calcium silicate fertilization with genetic resistance and fungicide spraying to control tan spot and FHB of wheat.

The effect of calcium silicate on tan spot severity increased from 2016 to 2018 due to increasing availability of the element in the soil, which increased the leaf concentration. The lower tan spot severity in 2016 was due to at least two factors: first, the absence or very low initial inoculum of *P. tritici-repentis* in the area because that was the first year that wheat was grown; second, the slower disease progress due a shortage

of rain during the stem elongation and booting stages. Under low disease pressure, the effects of treatments on disease mitigation were weak and more difficult to discriminate. However, the contribution of genetic resistance, calcium silicate amendment, and fungicide sprays were clear in both 2017 and 2018. The greatest difference in tan spot severity between $-Si$ and $+Si$ plants was observed for Tbio Toruk, the more susceptible cultivar, treated with fungicide at stem elongation. However, the lowest tan spot severity occurred in the Tbio Sossego plants treated twice with fungicide, especially in 2018, showing the importance of the high partial resistance to improve disease control. This result is in agreement with previous studies that showed higher reduction of tan spot by Si in plants with moderate resistance due to strong and fast defense activation (Dorneles et al. 2017, 2018; Pazdiora et al. 2018).

The change in the resistance components (previously reported by Dorneles et al. 2017; and Pazdiora et al. 2018) in the $+Si$ plants led to a smaller number and size of lesions and a longer time to complete the infection cycles by the pathogen, which thus reduced the progress of tan spot, resulting in lower disease severity at the end of the wheat cycle. There was a lower efficiency in the reduction of the disease by calcium silicate in the field compared to that reported for a greenhouse study (Pazdiora et al. 2018). However, it is important to highlight that although the effect in the field was lower than that in the greenhouse, it was still significant and significantly reduced the final tan spot severity and increased grain yield and quality.

Fungicide treatment, especially the second application at the flowering stage, was fundamental in $-Si$ plants to reduce the tan spot severity, particularly in the most susceptible cultivar, Tbio Toruk. However, the small difference in the severity of tan spot between $+Si$ plants with one fungicide spraying (stem elongation) and $-Si$ plants with two fungicide sprays (stem application and flowering), regardless of cultivar, indicates that the combination of calcium silicate fertilization with fungicide application had an additive effect on disease control. The results of this study show that calcium silicate amendment in the soil increased the effect of the intrinsic partial resistance of each genotype against the disease as well as the control obtained by fungicide application. The additive effect of calcium silicate in the soil with fungicide spraying has also been reported for the control of anthracnose of sorghum, and brown spot and blast of rice (Datnoff et al. 1991; Resende et al. 2013). Considering that in Brazil, control of disease in wheat typically involves at least two fungicide sprayings during the vegetative stages (Barro et al. 2017) and one to three applications in the reproductive stages (specifically for FHB control), totaling three to five applications during the crop cycle (D'Angelo et al. 2014), the results of this study indicate the possibility of reducing the number of fungicide sprayings required for tan spot control.

For FHB, the fungicide application at flowering had the most noticeable effect in reducing the disease incidence and severity. However, the moderate resistance of the cultivar Tbio Sossego to FHB was enhanced by calcium silicate, reducing the disease incidence when combined with fungicide application at the flowering stage. Furthermore, the addition of calcium silicate in the soil reduced the FHB severity and increased the efficacy of control conferred by the fungicide as indicated by the lower disease severity on $+Si$ plants. Infection by *Fusarium* occurs in the reproductive organs (Kang and Buchenauer 2000) through the penetration by hyphae in the anthers, in which the plant's defense mechanisms are inefficient against the pathogen (Kheiri et al. 2018). The moderate resistance observed in some wheat cultivars is associated with stronger plant defense through cell wall fortification (Walter et al. 2010; Ding et al. 2011) and increased biochemical defense mechanisms, restricting pathogen colonization (Pritsch et al. 2000; Walter et al. 2010; Ding et al. 2011). The results observed in the field, showing little or no effect of calcium silicate on FHB incidence but significant reduction of disease severity, mainly in 2017 and 2018, support the view that the effect of Si in plants acts mainly by enhancing the plant's defense mechanisms (Debona et al. 2017). However, for Si to initiate this effect on plant defense, a minimum concentration of the element in the plant tissue is needed (Dallagnol et al. 2011), which probably was not achieved in the 2016 growing season, as indicated by the lower concentration of Si in the soil and in the wheat tissues compared to the concentration observed in 2017 and 2018. Furthermore, the higher effect of Si on the control of tan spot than FHB may also be associated with a greater difference of Si concentration between plant organs, with the concentration being greater in the leaves than in the spikes.

The lower disease severity in $+Si$ plants was accompanied by increased grain yield of up to 1.0 ton per hectare in some combinations of cultivar and fungicide spraying, especially in 2017 and 2018. Furthermore, lower disease severity in $+Si$ plants resulted in increases, sometimes significant, in the 1000 grain weight and quality, measured by the hectoliter weight (always higher than the minimum of 70). However, the grain yield and quality were significantly influenced by the number of fungicide sprayings, being higher when plants were treated with fungicide at stem elongation and at flowering stages.

Although calcium silicate clearly reduced the severity of tan spot and FHB, the highest yields were always obtained with two fungicide applications. It was evident that Si confers benefits to wheat plants both in disease control and grain yield and quality. However, although Si significantly reduced the severity of FHB, the spraying of fungicide at the flowering stage is still essential for improved disease control. Thus, in order to obtain greater benefit from the effect of Si, aiming to reduce the number of fungicide applications (especially in the

vegetative phase), further studies should be conducted to evaluate the combination of different wheat cultivars with higher partial resistance, associated with crop rotation at one or two-year intervals, and realignment of fungicide application, especially in the vegetative phase.

5 Conclusions

The results of this study showed for the first time that the supply of calcium silicate to wheat plants was efficient in reducing the intensity of tan spot and FHB in the field. Furthermore, although the Si effect was significant, regardless of resistance level of the wheat cultivar or the number of fungicide applications, its supply to the plants with higher partial resistance resulted in lower intensity of tan spot and FHB.

The integration of the silicon improved grain yield and quality, showing that supply of calcium silicate to wheat plants is an alternative that can be integrated into wheat disease management programs, along with crop management practices and genetic resistance, to reduce the number of fungicide sprays.

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Code availability Not applicable.

Author contribution Conceptualization: L.J.D. and P.C.P.; Methodology: L.J.D., P.C.P. and K.R.D.; Investigation: P.C.P., K.R.D., and T.N.M.; Writing – original draft: L.J.D., P.C.P. and K.R.D.; Writing – review and editing: L.J.D., P.C.P. and P.N.; Resources: L.J.D., P.C.P. and K.R.D.; Funding acquisition: L.J.D.; Supervision: L.J.D. and P.C.P.

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

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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