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# Silicon potentiates biochemical defense responses of wheat against tan spot

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ABSTRACT

The silicon (Si) reduced tan spot (*Pyrenophora tritici-repentis*) severity up to 40%, through both biochemical defense mechanisms and histo-cytological defense responses. The activities of enzymes involved in plant defense system such as superoxide dismutase, peroxidase and chitinase showed greater activity in inoculated plants supplied with Si. Histo-cytological analysis indicated that Si potentiated the accumulation of hydrogen peroxide in the beginning of infection process. Together, these defense mechanisms resulted in the reduction of cell death at the infection sites in plants supplied with Si, which in turn showed lower disease severity. Si appears to be a promising alternative control measure for use in integrated management of tan spot.



## 1. Introduction

Tan spot, caused by *Drechslera tritici-repentis* (Died) Shoemaker, telomorph *Pyrenophora tritici-repentis* (Died.) Drechsler., is one of the most destructive leaf diseases of wheat (*Triticum aestivum* L.), able to reduce yield up to 48% [6]. Decrease in yield is mainly due to lower number and weight of grains, as well as their lower quality due red-rose pigmentation [27]. Yield reduction is associated with the negative impact of tan spot on the photosynthetically active leaf area due to the chlorosis and necrosis [18]. The fungus *P. tritici-repentis* is distributed worldwide in regions that grow wheat [17], and in Brazilian wheat growing areas, tan spot is considered the most frequent and damaging disease, especially when wheat is grown in areas with monoculture and no-till systems [6].

The symptoms of tan spot are initially observed on lower leaves near the soil. In the beginning, lesions start as small dark spots, which develop into elliptical lesions up to 12 mm in length, showing browning central necrosis with a chlorotic halo [18]. The intensity of both necrosis and chlorosis depends on the host's genotype and is associated with the amount of host selective toxins produced by the pathogen [3].

The role of these toxins in the pathogenesis of *P. tritici-repentis* in wheat is, when it entering the host cell, PtrToxA binds to a high-affinity receptor, yet unidentified, and then interacts in the chloroplast, causing changes in the photosystem I and II, resulting in both increases in the accumulation of reactive oxygen species (ROS) and inhibition of antioxidative enzymes, leading to cell death, which is visible as necrotic lesions in plant tissue [3,11]. The toxins PtrToxB and PtrToxC both cause chlorosis. After their infiltration into the host tissue, a process of photosynthesis inhibition begins, in ways still not well understood, resulting in the generation of ROS, thus causing chlorophyll photooxidation and finally chlorotic symptoms [18].

The alternatives for tan spot management are essentially based on crop practices aiming to reduce the initial inoculum, such as rotation and the use of pathogen-free seeds and foliar spraying of fungicides such as triazoles and strobilurins [2]. This chemical control with fungicides has shown satisfactory efficiency in most situations [19]. However, there are reports of the occurrence of resistance to fungicides of the triazole and strobilurine groups [12]. The use of resistant cultivars would be a more efficient control measure against *P. tritici-repentis*, but so far this is not an alternative available to farmers [17]. Thus, in view of the potential damage of tan spot and the limited availability of effective measures for its management, the search for new control strategies that complement the effect of reducing the initial inoculum and increasing

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wheat resistance to tan spot is fundamental.

A control strategy with great potential for integrated tan spot management is the use of silicon (Si), which has been shown to be effective in reducing the intensities of several diseases in crops with high economic importance [24]. Several studies have shown that Si supply to various plant species (mono- and di-cotylendons) through the soil, leaves or in nutrient solutions, significantly reduces the intensity of many important diseases [24]. However, the best results in disease intensity reduction have been obtained when Si is present in the soluble form within the plant tissue, which occurs when the element is taken up by the root system. Numerous host defense mechanisms against pathogens enhanced by Si have been reported, such as enhancement of the phenylpropanoid pathway, which increases the concentration of phenolics and lignin and produces diterpenoid phytoalexins; papilla formation at the infection sites; changes in the activities of enzymes such as chitinases (CHI), peroxidases (POX), phenylalanine ammonia lyases (PAL), lipoxygenases (LOX); and changes in the plant primary metabolism [22,23,32].

In wheat, a high foliar Si concentration reduced the severities of powdery mildew (*Blumeria graminis f.sp. tritici*) [1,21], spot blotch (*Bipolaris sorokiniana*) [8] and blast (*Pyricularia oryzae*) [31,34]. The wheat resistance to *B.* graminis f. sp. *tritici* conferred by Si was associated with an increase in the formation of papillae and the concentration of phenolics and lignin and an increase in the activities of superoxide dismustase (SOD) and POX [21]. For the wheat-*B. sorokiniana* pathosystem [8], there was an increase in the activities of CHI, POX and higher lignin concentration in plants supplied with Si. The enzyme glutathione reductase has been found to be essential to limit the oxidative stress caused by *P. oryzae* infection in wheat plants supplied with Si [7].

In this context, the hypothesis of the present study is that Si can possibly enhance the defense responses (activities of chitinases (CHI) and oxidoreductases (SOD, CAT, POX, PPO and LOX)), lignin concentration, *in situ* accumulation of hydrogen peroxide and cell reaction against fungal infection of wheat plants against *P. tritici-repentis* infection, thus reducing the symptoms of tan spot. To test this hypothesis, wheat plants of a moderately resistant and a susceptible cultivar to tan spot were supplied or not with Si and inoculated or not with *P. tritici-repentis*.

## 2. Material and methods

### 2.1. Plant material and growth

The wheat cultivars Fundacep Horizonte (CCGL TEC/FUNDACEP) and Quartzo (OR Sementes), susceptible and moderately resistant to tan spot [4], respectively, were used in the experiment. Seeds were sowed in four lines (two for cv. Fundacep Horizonte and two for cv. Quartzo) in 20 L plastic trays (0.6 m long × 0.38 m wide × 0.15 m deep) containing 19 kg of soil (Supplementary S1). Plants were kept inside a greenhouse during the experiment, with daily mean temperature ranging between 15 °C and 25 °C.

### 2.2. Soil characteristics and treatment amendments

The soil used in the experiment was collected in the experimental area of Federal University of Pelotas in the city of Capão do Leão, Rio Grande do Sul, with the following physicochemical characteristics: 251 g $kg^{-1}$ of clay; 54 g $kg^{-1}$ of silt; pH $CaCl_2$ (0.01 M) = 4 1; P (resin), S, Mn, B, Cu, Fe, and Zn = 12, 6, 1.5, 0.24, 0.5, 329 and 1.28 mg $dm^{-3}$ respectively; K (resin) = 1.9 mmolc $dm^{-3}$; $Al^{3+}$, $Ca^{2+}$, $Mg^{2+}$, H + Al and CTC = 17, 5, 4, 71 and 81.7 mmolc $dm^{-3}$, respectively; base saturation = 12%; organic matter = 14 g $dm^{-3}$. The concentration of available Si (extracted with 0.01 M $CaCl_2$) was 6.0 mg $dm^{-3}$ determined according to Korndorfer et al. [16]. The soil fertility was corrected using chemical fertilizer to achieve a yield of 4.0 ton $ha^{-1}$ [4].

The source of Si was calcium silicate (Agrosilício®, Agronelli Insumos Agrícolas, Uberaba, Brazil), which is composed of 10.5% Si, 25.0% calcium and 6.0% magnesium. The product was mixed with the soil at a rate of 8.21 ton $ha^{-1}$ 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 mixed with extra-fine limestone at the rate of 6.48 ton $ha^{-1}$. The extra-fine limestone (Dagoberto Barcelos, Caçapava do Sul, Brazil) was composed of 26.5% calcium and 15.0% magnesium. Calcium carbonate (Synth, Diadema, Brazil) and magnesium carbonate (Synth, Diadema, Brazil) were used to adjust the concentration of calcium and magnesium, respectively. After complete soil homogenization in each treatment, water was added to reach at 80% of field capacity, followed by 30 days of incubation in plastic bags.

### 2.3. Experimental design

The eight treatments were arranged in a fully randomized 2 × 2 × 2 factorial design where the factors consisted of cultivars (Fundacep Horizonte or Quartzo), application of Si (with or without application) and plant inoculation (inoculated with *P. tritici-repentis* or sprayed with water (mock-inoculated)) with four replications. The experiment was performed twice.

### 2.4. Fungal growth, inoculation procedure and disease evaluation

An isolate of *P. tritici-repentis* (LIPP 0113) classified as race 1 [26], was obtained from a commercial wheat crop (cultivar BRS Guamirin) and used in the experiments. The fungal growth was performed according to the method described by Ranzi and Forcelini [19]. Briefly, the growth media used was V8 composed of 3.0 g $L^{-1}$ of calcium carbonate (Synth, Diadema, Brazil), 150 mL $L^{-1}$ of juice of eight vegetables (Campbell's V-8®, Camden, USA), 15 g $L^{-1}$ of agar (Kasvi, Curitiba, Brazil) and distilled water. After fungal growth in the V8 media for five days in darkness at 25 ± 1 °C, the fungal colony was stressed by mycelium scraping. Then, the fungal colony was exposed for 24 h to light (Osram®, 40w) at 25 ± 1 °C, to allow development of conidiophores, followed by a further 24 h of darkness at 15 ± 1 °C, necessary for conidia formation. At the end of seven days, the conidia were collected in sterile water containing 0.01% Tween 20 and the concentration was adjusted to 6 × $10^3$ conidia $mL^{-1}$. To aid in conidial adhesion on leaf surfaces, 1% neutral gelatin (p/v) was added to the suspension before inoculation. This suspension was sprayed onto leaves of 40-day old wheat plants (growth stage 8; Large, 1954) as a fine mist using a hand-held manual sprayer (Tecblas, REF: 359–60 mL/Porto Alegre, Brazil) until runoff. Immediately after inoculation, the plants were transferred to a humid chamber with relative humidity of 90± 5%, temperature of 25 ± 3 °C and photoperiod of 12 h. After 48 h in the humid chamber, the plants were transferred to an environment with relative humidity around 70%, temperature of 25 ± 2 °C and photoperiod of 12 h. Mock-inoculated plants were sprayed with distilled water and exposed to the same conditions of inoculated ones.

The following resistance components were evaluated on the third and fourth leaves of each plant: incubation period (IP), relative infection efficiency (RIE), rate of lesion expansion (*r*), final lesion size (FLS), final number of lesion (FNL) and final severity (FS). The IP (hours) was assessed by examining the leaves every three hours after inoculation using a magnifier with 20 × amplification