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# Silicon treatment in oil palms confers resistance to basal stem rot disease caused by *Ganoderma boninense*

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ABSTRACT

Basal stem rot (BSR) disease, which is caused by the fungus *Ganoderma boninense*, is the major disease of oil palm in Malaysia and causes economic losses in the oil palm industry around the world. Plants that are treated with silicon (Si) show enhanced host resistance, perhaps because the accumulation of silica in host cell walls deters the pathogen from penetrating host tissues. In this study, oil palm seedlings were treated with five Si sources (silicon oxide, potassium silicate, calcium silicate, sodium silicate, and sodium meta-silicate) at four concentrations (0, 800, 1200, and 2000 mg $L^{-1}$) to evaluate the effects of Si treatment on the growth and resistance to *G. boninense* of oil palm. Treatment played a role in keeping the *G. boninense* infection below the threshold for BSR initiation by restricting the fungus from entering and traveling through host tissues, as assessed by foliar symptoms and examinations of the root and bole for infection. At eight months after inoculation, palms in the control group, which had received no supplemental Si fertilizer, demonstrated the highest levels of disease severity, with estimated 95% cell damage and high physiological stress caused by *G. boninense*. Inoculation of seedlings with $SiO_2$ at a concentration of 1200 mg $L^{-1}$ was most effective in suppressing BSR and provided a 53% disease reduction compared with other treatments. Silicon nutrition also reduced the numbers of primary roots infected and of stem tissues that developed lesions.



## 1. Introduction

There have been considerable developments in oil palm *(Elaeis guineensis* Jacq.) cultivation during the past 20 years in Malaysia. Oil palm cultivation began in 1917, but the industry's growth was initially very slow. Within the past four decades, there has been a rapid expansion of the acreage planted to oil palm. As of December 2013, the total area planted with oil palm in Malaysia was 5,229,739 ha, belonging to private estates (62%), independent smallholders (14%), Federal Land Development Authority (FELDA) (13%), state schemes and government agencies (6%), Rubber Industry Smallholders Development Authority (RISDA) (2%), and Federal Land Consolidation and Rehabilitation Authority (FELCRA) (3%) (Malaysian Palm Oil Board, 2013).

Diseases substantially reduce the yearly harvest, particularly basal stem rot (BSR) disease, which significantly lowers the fresh fruit bunch yield of oil palm. BSR is caused by a fungus, *Ganoderma boninense*. The fungus typically infects seedlings in one to two years after they are planted and increases in four-to five-year-old palms (Ariffin et al., 2000). A survey on BSR in oil palm conducted by the MPOB in 2009–2010 showed that the disease incidence in Malaysia, including Peninsular Malaysia, Sabah, and Sarawak, was 3.71% and 59,148 ha were affected (Idris et al., 2011). The BSR symptoms include a mottling or yellowing of fronds, followed by necrosis. Spear leaves eventually remain unopened. It is assumed that at least half of the basal stem has been killed by the fungus by the time foliar symptoms are observed (Idris et al., 2000). The tree eventually collapses, leaving diseased bole tissue in the ground. A major factor that spread the disease was the practice of replanting a new oil palm in the same area that was infected by BSR disease.

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*G. boninense* is soil borne and has a resting stage in which it is inactive in the soil, followed by an attack on the host.

Detecting *G. boninense* infection early allows palms to be treated, avoiding more extensive damage to the tree. Some BSR disease control methods in existing stands and management strategies related to replanting have been developed and are being implemented in several oil palm plantations and smallholders in Malaysia. A normal practice in many countries is to destroy the diseased oil palm by destroying the diseased roots, stump, and trunk of an infected oil palm. The protocol is to remove the diseased palm by digging out the stump, root, and underlying soil to a specific depth, refill the hole with nearby soil, and plant a new palm (Idris et al., 2005). An oil palm breeding program also was developed with the goal of preserving genetic material, protecting genetic diversity, and selecting favorable traits in oil palm that confer resistance to *G. boninense*. Chemical controls were also used successfully to prevent the growth of *G. boninense*. The application of hexaconazole also improved oil palm productivity, prolonging the life of BSR-affected palms (Idris et al., 2002).

Due to the limitations of chemical pesticides, it seems appropriate to seek an alternative control strategy that is more environmentally friendly. Manipulation of nutrient uptake is an important alternative strategy, as all essential plant nutrients influence the health of plants and their susceptibility to disease (Agrios, 2005).

Silicon (Si) is not recognized as an essential element for plant growth (Epstein, 1999), but the beneficial effects of this element on growth, development, yield, and disease resistance have been observed in a wide variety of plant species (Adatia and Besford, 1986; Hazama et al., 1993; Miyake and Takahashi, 1983; Rodrigues et al., 2001). Silicon fertilizers are routinely applied to several crops, including rice and sugarcane, to provide high and sustainable crop yields. The beneficial effects of Si are mainly associated with its high level of deposition in plant tissues, enhancing their strength and rigidity (Takahashi, 1987). Silicon may play an active role in enhancing host resistance to plant diseases by stimulating defense reaction mechanisms. Little is known about *G. boninense* disease etiology and the epidemiology of oil palm diseases; it is necessary to develop a greater understanding of the basic biochemical processes underlying disease. In addition, if Si supplementation proves to be a viable alternative treatment for BSR, Si must be studied further to identify the multiple modes of its action and potential in order to tackle the problem of oil palm disease. Therefore, the present study has been designed to examine the ability of Si to suppress *G. boninense* disease.

## 2. Material and methods

### 2.1. Materials and experimental design

This study was conducted at experimental field 2 of Universiti Putra Malaysia. The temperature was 28–35 °C, light intensity was 280–340 μmol $m^{-2}$ $s^{-1}$, and humidity was 80–88% at the site. Germinated seeds of oil palm were collected from FELDA, Perak, Malaysia. The germinated seeds were planted in small, black, polypropylene bags (6 × 9 cm) for 3 months in a greenhouse and then transferred to large, white, polypropylene bags (15 × 23 cm) containing a 3:2 mixture of sterilized top soil and organic matter. Soil was analyzed for physical and chemical properties using the Kjeldahl method, and soil organic carbon was determined according to Walkley and Black (1934). Available phosphorus was determined by Murphy and Riley method (Bray and Kurtz, 1945) and exchangeable K, Ca, Mg, and Na were determined by the ammonium acetate extraction method (Benton Jones, 2003). A factorial experiment was conducted with 10 seedlings per replication and arranged in a completely randomized design.

### 2.2. Treatments and fertilizer application

Silicon was applied as silicon oxide, potassium silicate, calcium silicate, sodium silicate, and sodium meta-silicate. The control plants received no application of Si. At first, 10,000 mg $L^{-1}$ of each source of soluble Si (silicon oxide, potassium silicate, sodium silicate, and sodium meta-silicate) were prepared separately as a stock of Si treatments. Appropriate amounts from these stocks were used to generate three treatments with different concentrations of Si (800, 1200, and 2000 mg $L^{-1}$), calculated on the basis of molecular weight. Each polypropylene bag was then drenched with Si by pouring into it 500 mL of the 800, 1200, or 2000 mg $L^{-1}$ solution. Meanwhile, the treatment with non-soluble calcium silicate was prepared in the amounts of 2.11 g, 4.22 g, and 7.40 g, equivalent to 800, 1200 and 2000 mg $L^{-1}$, respectively, and added to the growing medium. All seedlings received the Si treatments at a monthly interval for five months. During the experiment, the oil palm seedlings received regular watering and applications of manure, in addition to applications of pesticides as appropriate. Nutrient supplement was added according to the methods of Rankine and Fairhurst (1998).

### 2.3. Preparation of G. boninense culture

The slant culture of *G. boninense* (Isolate PER 71) was collected from the MPOB. It was then sub-cultured on potato dextrose agar (PDA) at 28 °C for 8–9 days until the mycelium fully covered the PDA. The PDA medium was prepared by dissolving 19.5 g of PDA powder in 500 mL distilled water and then sterilized by autoclaving at 121 °C for 20 min. The *G. boninense* culture was examined daily to observe the morphology and growth of mycelia and to inspect for bacterial or fungal contamination. Cultures with bacterial or fungal contamination were discarded. Cultures that formed a crust after 6 or 7 days were considered to be good cultures and were used to prepare *G. boninense* rubber wood block (RWB) inocula for oil palm infection.

### 2.4. Preparation of G. boninense rubber wood blocks

Rubber wood blocks (RWBs) of 2.5 cm × 2.5 cm × 5 cm were used as a medium on which the *G. boninense* inocula were prepared. The RWBs were washed and placed in propylene bags containing 2% malt extract (which had been incubated for 12 h) and autoclaved for 1 h at 121 °C. After sterilization and cooling were complete, the RWBs in the polypropylene bags were inoculated with *G. boninense*. *G. boninense* culture was used to inoculate each RWB by cutting into small pieces and transferring them all to the surface of the RWB. The propylene bags were then tied up neatly with rubber bands, and aluminum foil was used as a cap. All work was done under laminar flow conditions to avoid any contamination. Then, the RWBs were incubated in the sealed box (at 27 °C) for 30–60 days. Fully colonized RWBs were used for inoculation.

### 2.5. Inoculation of seedlings with G. boninense

The inoculation technique that follows has been described by Ariffin and Idris (1990). Inoculation was carried out by inserting fully colonized RWBs among the roots of an oil palm seedling to ensure close contact between the mycelia and oil palm roots. The inoculated seedlings were then planted in polythene bags (15 × 23 cm) containing a soil mixture (an equal amount of top soil and organic matter).