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Research paper

## Root-applied silicon in the early bud stage increases the rapeseed yield and optimizes the mechanical harvesting characteristics



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#### ABSTRACT

A liquid silicon fertilizer [Si(OH)4] was root sprayed at various concentrations during the seedling stage (T1) and early bud stage (T2) using the rapeseed varieties (Brassica napus L.) Huayouza 62 and Fengyou 520. The lodging- and yield-related index, silique shatter were evaluated. Our results indicated that the lodging resistance, silique shatter resistance, and yield all increased significantly in the two rapeseed varieties after the application of silicon fertilizer in both stages. The seed number per silique was reduced after silicon application, but the silique number per plant and 1000-grain weight were increased, especially the silique number on the branches, thereby improving the overall yield, where the most obvious effect on the yield increase occurred at the T2 stage with 0.96 mM silicon. At the T1 and T2 stages, the higher concentration of silicon fertilizer increased the root diameter, as well as the stem SiO2 content and breaking strength, but reduced the plant height and lodging index. There were also increases in the expression levels of the key genes related to stem lignin biosynthesis (phenylalanine ammonia lyase (PAL) and 4-coumarate: CoA ligases), the lignin content, thereby increasing the resistance to lodging. At stages T1 and T2, the higher concentration of silicon fertilizer increased the expression of the PAL, which induced the accumulation of lignin. There was a significant correlation between the silique dry weight and silique shatter index. The silique SiO2 content and dry weight also increased, thereby improving the shatter resistance. Considering the yield and mechanical harvesting properties, the results of the present study show that the optimum effect of the liquid silicon fertilizer on both rapeseed varieties occurred during the early bud stage (bolting height of 8-12 cm) with a monosilicic acid content was 0.96 mM.

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#### 1. Introduction

Rapeseed is one of the world's four main oil crops and the fifth largest crop in China, where the annual planting area is 7.52 million hectares (FAO, 2014). However, at present, the mechanized area is small in China and mechanical harvesting only accounts for 30.8% of the mechanized farming area (China's agricultural mechanization statistical yearbook, 2014), which is mainly due to the low efficiency of mechanized harvesting and the high yield loss rate. High yield cultivation is a prerequisite for obtaining a high mechanical harvesting yield, but the excessive application of nitrogen to obtain high yields often leads to the occurrence of rapeseed lodging and silique shatter, which increases the loss rate during mechanical harvesting. Therefore, in addition to a high yield, the

\* Corresponding author. E-mail address: zhougs@mail.hzau.edu.cn (G. Zhou). mechanical harvesting properties of rapeseed should meet the criteria for high lodging resistance and high silique shatter resistance (Hua et al., 2014).

Silicon is the fourth major nutrient element in gramineous crops, following nitrogen, phosphorus, and potassium. Its concentration in the soil solution varies from 0.1 to 1.4 mM and plants absorb it as silicic acid [Si(OH)<sub>4</sub>] at a pH below 9 (pKa = 9.8) (Marschner, 1995). As a consequence, all plants grown in soil contain some Si in their tissues. However, repeated cropping and the application of chemical fertilizers deplete the amount of Si that is available to plants (Ma and Yamaji, 2006). Silicon deficiency in soils is now recognized as a limiting factor for crop production, especially for Si-accumulating plants such as rice and sugarcane, and Si fertilizers are routinely applied to enhance the yields of these crops (Ma and Yamaji, 2006). The deposition of Si protects plants from multiple abiotic and biotic stresses (Ma and Takahasi, 2002; Ma and Yamaji, 2006). Si can increase the resistance of plants to pathogens and insects (Fauteux et al., 2005), as well as against chemical (e.g.,

nutrient imbalance and salt) and physical stresses (e.g., freezing, lodging, radiation, high temperature, and drought) (Ma, 2004; Ma and Yamaji, 2006).

The effect of Si application on the yield has been studied in different plants, such as rice (Oryza sativa L.), a typical Si active accumulator; cucumber (Cucumis sativus), which is, among other dicots, the one that accumulates higher Si concentrations in shoots (Liang et al., 2005a,b) and tomato (Lycopersicon esculentum), that accumulates low levels of Si (Mitani and Ma, 2005). The application of silicon fertilizer to rice significantly increases the grain number per panicle but there is no substantial effect on the 100grain weight and panicle number, so the yield increases (Deren et al., 1994). Silicon-fertilized cucumber plants have dark green leaves and the leaf thickness is increased. The dry weight per unit area is also increased, which delays leaf senescence (Adatia and Besford, 1986). The application of silicon can also improve the yield in tomato, although its silicon absorption capacity is low (Mitani and Ma, 2005). Gramineous crops have a greater capacity for silicon absorption, and thus studies of the effects of silicon fertilizer on lodging resistance have been common in gramineous crops. It has been demonstrated that the application of silicon decreases the base internode length in rice, increases the base diameter, and enhances the breaking strength of the base internode, thereby improving lodging resistance in rice.

Lignin is the main component of the secondary cell wall and it can increase the mechanical strength of the stem, which lowers the risk of lodging in crops (Marie et al., 1998), and thus its content significantly affects crop lodging (Jones et al., 2001). Phenylalanine ammonia lyase (PAL) catalyzes the first step of the lignin synthesis pathway (Korth et al., 2001), the cinnamoyl-CoA reductase (CCR) enzyme catalyzes the conversion of cinnamoyl-CoAs to cinnamaldehydes in lignin biosynthesis (Rogers and Campbel, 2004), and 4-coumarate: CoA ligases (4CL) are key enzymes in the monolignols biosynthetic pathway, which is an important complex aromatic polymer for lignin biosynthesis (Hai et al., 2003). It is widely accepted that normal plant growth depends on the strict regulation of these genes at specific times and in specific tissues. The activity of PAL increases with the addition of silicon fertilizer (Liang et al., 2005a,b,c), which is beneficial for lignin accumulation. However, other studies have shown that the expression levels of PAL and CCR1 are elevated in rice leaves without silicon application, and the cell wall is thickened (Yamamoto et al., 2012).

Thus, some studies have investigated the effects of silicon application on gramineous crops, but few in rapeseed. In the present study, under high yield cultivation conditions, we treated two rapeseed varieties with distinct characteristics in terms of the yield, lodging resistance, and shattering resistance using a fertilizer solution containing different silicon concentrations, which was sprayed on the roots during two developmental stages. We assessed the effects of silicon application on the yield, lodging, and silique shatter in rapeseed, as well as its effect on the lignin content and lignin biosynthesis genes to understand the mechanism related to the effects of the silicon fertilizer. Our results may provide a technical and theoretical basis for the establishment of high yield rapeseed varieties that are suitable for mechanical harvesting with reasonable application rates of silicon fertilizer.

#### 2. Material and methods

#### 2.1. Experimental design

The experiments were performed at the experimental site of Huazhong Agricultural University (N30.52 $^{\circ}$ , E114.31 $^{\circ}$ ) during 2013–2014 and 2014–2015. The rapeseed was sown on 09/21/2013 and 09/26/2014, where the seedling density was evaluated directly

after emergence and adjusted to ensure a precise planting density of  $45 \times 10^4$  plants  $ha^{-1}$  at the five-leaf growth stage. The dimension of each plot was  $10 \times 2 \, \text{m}$  and pure nitrogen were applied at 360 kg ha<sup>-1</sup> to each plot. Urea was used as the nitrogen source and the base fertilizer, seed bed fertilizer, and bud fertilizer were applied at a ratio of 5:2:3. The combined concentration of applied phosphorus fertilizer (P2O5) and potassium fertilizer  $(K_2O)$  was 150 kg ha<sup>-1</sup>, which were provided as calcium superphosphate and potassium chloride, respectively. Borax was utilized at a concentration of 7.5 kg ha<sup>-1</sup>. Phosphorus, potassium, and boron fertilizer were all applied once as base manure. The previous crop in the experimental field was rice, which was harvested in early September. The two-year soil conditions were as follows. In 2013, the soil alkali-hydrolyzable nitrogen, available phosphorus, and available potassium contents were  $86.72 \,\mathrm{mg \, kg^{-1}}$ ,  $14.92 \,\mathrm{mg \, kg^{-1}}$ , and  $145.49 \,\mathrm{mg\,kg^{-1}}$ , respectively. In 2014, the concentrations of these three elements were  $80.77 \,\mathrm{mg\,kg^{-1}}$ ,  $13.72 \,\mathrm{mg\,kg^{-1}}$ , and  $140.04 \,\mathrm{mg}\,\mathrm{kg}^{-1}$ , respectively.

Two rapeseed varieties with distinct characteristics in terms of the yield, lodging resistance, and shattering resistance were selected for use as the experimental materials: swede-type rape hybrid Huayouza 62 and Fengyou 520 (the lodging resistance and silique shatter resistance of Huayouza 62 are higher than those of Fengyou 520, and the yield of Fengyou 520 is higher than that of Huayouza 62) (data can be seen in Kuai et al., 2015). A split plot design was employed with the tested varieties in the primary plots and the stages for spraying as the subplot (seedling stage T1 with 7–8 green leaves, leaf area index = 3.0–3.5; early bud stage T2, with bolting height = 8-12 cm and leaf area index = 4.9-5.4), as well as basic solution with 0.48 mM (P1), 0.96 mM (P2), and 1.44 mM (P3) monosilicic acid  $[Si(OH)_4]$  as the sub-subplot. 750 Lha<sup>-1</sup> of the solutions were sprayed to each plot. The seedling stage and the early bud stage were selected for application times in an effort to most effectively improve leaf quality and control stem development, respectively. The same amount of water was used as the control (P0) for both developmental stages. The root system was sprayed with three biological replicates for each plot.

#### 2.2. Measurements and methods

#### 2.2.1. Yield and yield components

Plots were harvested when approximately 2/3 of the seeds were brown. Ten plants were randomly sampled in each plot by slowly uprooting, where the taproot and large lateral roots were retained. Next, the yield components and seed yield per plant were determined. For each plant, the following measurements and observations were made: plant height (cm), number of siliques per plant, number of seeds per silique, and 1000-seed weight. Plant tissue samples were separated from the cotyledonary node into the roots and aboveground tissues. After determining the fresh weight, the roots and aboveground tissues were dried in an oven for 30 min at 105 °C to deactivate enzymes, and then dried again at 80 °C until constant weight to determine the dry weight.

#### 2.2.2. Lodging related indexes

The snapping resistance and lodging resistance index were measured at maturity according to the methods described by Wang et al. (2014), with some modifications. The snapping resistance of 10 cm of the basal stem was measured with a plant lodging tester (YYD-1, Hangzhou TOP Instrument Co. Ltd, Hangzhou, China). The tester was set perpendicular to the stem at the middle and loaded gradually, where the snapping resistance was measured when the stem internode broke. The value displayed was the breaking strength in kg. The stem lodging resistance index was calculated using the following equation (Wang et al., 2014):

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