Management of ratoon stunting disease by hot water treatment to provide healthy cane seed

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Abstract. Ratoon stunting disease (RSD) is one of major sugarcane diseases in Indonesia. The causal agent of the disease is Leifsonia xyli subsp. xyli. The pathogen systemically infects xylem vessel. Providing healthy seed cane is the most important management of the disease. This study aimed to determine the best Hot Water Treatment (HWT) method for reducing RSD incidence of sett cane. There were eight treatments arranged in randomized blocked design, i.e.: Bud sets were (1) soaked in running water (RW) for 48 h and then treated with 50°C HWT for 2 h; (2) RW for 5 h and 50°C HWT for 2 h; (3) directly 50°C HWT for 2 h; (4) directly 45°C HWT for 30 m; (5) soaked in bactericide suspension for 2 h; (6) directly planted without any treatment; (7) RW for 48 h; (8) RW for 5 h. Results of the study showed that germination was only 23-27% when treated with 50°C HWT, but incidence of RSD was only 3-6%. Germination increased when soaked in running water for 5 h before HWT. The fastest bud germination occurred at 45°C HWT for 30 m with the highest percentage of germination (83%) but incidence of RSD was high.

1. Introduction

One of important diseases on sugarcane is ratoon stunting disease (RSD), caused by Leifsonia xyli subsp. xyli (Lxx) [1]. Infection started from diseased seed cane, hence plant cane (PC) and ratoon cane (RC) could suffer from this disease. However, yield reduction is more significant in RC. RSD was first found in 1960 at sugarcane plantation in Java, but then became serious in 1970’s and infected almost 55% of sugarcane plantation in Java [2]. Yield loss ranged 5-10%, and could increase up to 30% in ratoon crop. Grisham [3] stated that yield decreased up to 14% in plant cane (PC) and 27% in ratoon cane (RC1). At the field, infected crops are almost symptomless, unless in ratoon crops the internode are shorter than usual normal plant. RSD is categorized as a systemic disease since the pathogen colonizes and develops throughout the xylem vessels of the plant and spreads through the infected setts for the next crop [4] and also through contaminated tools [5]. Therefore, providing healthy setts is the most important factor to manage the disease for the next season.

Disinfecting the knife use for cutting setts with benzalconium chloride 0.1% or didecyldimethyl-ammonium chloride for 5 minutes could reduce the number of bacterial contaminations [6]. Treat the set with hot air, moist air, aerated steam or hot water to eliminate Lxx are recommended [7]. In USA, infected sets were treated with hot water 52°C for 20 minutes, followed by another hot water treatment 52°C for 20 minutes for the next day to kill bacteria and insects.
However, Hot Water Treatment (HWT) reduced bud germination and sometimes killed the buds. In Australia, sets were soaked in water for 40-48 hours before HWT 50 °C for 3 hours to control *Lxx* and *Xanthomonas albilineans* helped improving bud germination.

This trial aimed to observe the effect of HWT on the severity of RSD and also bud germination and other cane growth factors.

2. Materials and methods.
The research has been conducted for two years (plantcane/PC and ratooncane/RC) using Lxx infected BQ1300 variety. The treatments were as follow: (1) Bud sets were soaked in running water for 48 h and then were treated with hot water 50°C for 2 h; (2) Bud sets were soaked in running water for 5 h and then were treated with hot water 50°C for 2 h; (3) Bud sets were directly treated with hot water 50°C for 2 h; (4) Bud sets were directly treated with hot water 45°C for 30 minutes; (5) Bud sets were soaked in 0.2% bactericide (streptomycin sulphate) suspension for 2 h; (6) Control (the buds were planted without any treatment); (7) Bud sets were soaked in running water for 48 h; (8) Bud sets were soaked in running water for 5 h.

Observations were made for: (1) Agronomical aspects (percentage of germination, number of tillers, number of internodes, plant height, and stalk diameter); (2) RSD severity: number of infected stalks divided by number of tillers in one clump. Estimation was done when the plants were 8 months old.

3. Results and discussion

3.1. Effect of HWT on Sugarcane Agronomical Aspects
HWT significantly affected percentage of germination and number of tillers. The best germination was obtained when sets were treated with hot water 45°C for 2 h. The buds germinated 83% homogeneously two weeks after planting. HWT 50°C for 2 h delayed germination buds and caused poor germination, particularly when sets were soaked in running water for 48 h or directly put in HWT the buds (Figure 1).

![Figure 1](image-url)

**Figure 1.** Development of bud germination of all the treatments; 1. Running water 48 h + HWT 50°C 2 h; 2. Running water 5 h + HWT 50OC 2 h; 3. HWT 50 OC 2 h; 4. HWT 45 OC 30 m; 5. Streptomycin sulphate 0.2% 2 h; 6. Directly plant; 7. Running water 5 h; 8. Running water 48

Number of tillers of all of the treatments in PC ranged between 10.4 and 13, and then slightly decreased in RC except when sets were HWT 50°C for 2 h the number was only 5.9 in PC and increased to 9.39 in RC (Table 1). As [8] stated that RSD reduced stalk length and diameter as well as number of tillers.
Measurement of internode length and plant height was done at 8 month old plant. The longest internode appeared in plant cane treated with HWT50°C for 2 h directly or soaked in running water for 5 or 48 h and also treated with bactericide. Generally, plant height and internode length were shorter in RC than those in PC (Figure 2).

Figure 2. Plant height and Internode length of plant cane (PC) and ratoon cane (RC) in various treatments; Number of the treatments is the same as Figure 1.

| Treatment                               | Germination (%) | Number of Tillers |
|-----------------------------------------|----------------|-------------------|
| Running water 48 h + HWT 50°C 2 h      | 23.3 d         | 10.4 ab           |
| Running water 5 h + HWT 50°C 2 h      | 63.3 bc        | 12.0 a            |
| HWT 50°C 2 h                           | 26.7 d         | 5.9 b             |
| HWT 45°C 30 m                          | 83.3 a         | 12.0 a            |
| Streptomycin sulphate 0.2% 2 h        | 63.3 bc        | 12.1 a            |
| Directly plant                          | 80.0 ab        | 11.7 a            |
| Running water 5 h                      | 73.3 abc       | 12.4 a            |
| Running water 48 h                     | 63.3 c         | 13.0 a            |
3.2. Effect of HWT on Ratoon Stunting Disease (RSD) Incidence

RSD did not give external symptoms clearly, except in RC the growth slightly decreased. However, when the stalk was longitudinally sectioned, the infected plant showing red to brownish red discoloration of the nodes (Figure 3.).

![Healthy cane](image1.png) ![Diseased cane](image2.png)

**Figure 3.** The difference between healthy cane (left) and diseased cane with RSD symptom (right)

HWT 50°C for 2 h directly or soaked in running water 5 h or 48 h before it, reduced RSD incidence significantly (3-15%). HWT 45°C for 30 m or soaking setts in 0.2% bactericidal suspension for 2 h could not eliminate RSD, although the incidence was lower (59% and 45% respectively) than sugarcane plants from setts without any treatments (70%). Setts soaked in running water before planting for 5 or 48 h tended to increase RSD incidence (Figure 4).

RSD incidence increased in RC, with the highest incidence occurred in RC treated with bactericide (98%) (Figure 4). The lowest RSD incidence was still in sugarcane treated with HWT 50°C for 2 h directly or soaked in running water for 5 h or 48 h before it and the percentage was almost the same as PC (6-10%).

![RSD Incidence (%)](image3.png)

**Figure 4.** RSD Severity on Various HWT; Number of the treatments is the same as Figure 1.

Direct HWT 50°C for 2 h negatively affected bud germination and number of tiller. Numerous eye buds were injured during HWT 50°C for 2 h. Soaking setts in running water for 5 h followed by HWT 50°C for 2 h showed better results, although, the survived buds germinated lately, but they grew normal and produced tillers as much as other treatments. This finding was different to the previous results [9]. Setts were treated with HWT 45, 50, or 52°C showed increasing in germination, plant height, diameter of stalk, and yield [9].

Furthermore, HWT 50°C for 2 h either dipping or not in running water was able to reduce RSD incidence significantly. HWT 45°C for 30 m did not affect germination, growth, or production of tiller,
but the treatment could not eliminate RSD incidence. Dipping the setts in 0.2% bactericidal suspension did not help reducing RSD incidence, even in RC the incidence increased.

3.3. Correlation Between Plant Growth and Disease Incidence

External symptom of RSD was more obvious in RC. The early performance of 2 month old of RC showed that RSD started affecting the plant growth (Figure 5). This situation was supported with evidence that the disease incidence was negatively correlated with plant height (Figure 6) and internode length, particularly in ratoon cane (Figure 7). Negative correlation means that high disease incidence would reduced plant growth.

![Figure 5](image_url)

**Figure 5.** Performance of 2 month old RC in various treatments; Number of the treatments is the same as Figure 1.

![Figure 6](image_url)

**Figure 6.** Correlation Between Disease Incidence and Plant Height in Plant Cane (a) and Ratoon Cane (b)

This result corresponds with [10] and [11] studies. RSD incidence in PC from bud setts treated with hot water 50°C for 2 h was only 8%, compared to untreated setts (77%) [10]. Lxx could be controlled by HWT 50°C for 2 h, but the pathogen still remained in the crop [11]. Successful control of RSD in sugarcane plantation in Australia also occured when seed cane was treated with hot water. The incidence of RSD was less than 5%, even in some areas were only 1% [12].
Figure 7. Correlation Between Disease Incidence and Internode Length in Plant Cane (a) and Ratoon Cane (b)

Addition of HCl (1%) or CH₃COOOH (2%) + SDS (1%) for 10 minutes gave better control and reduced the negative effect of heat on bud germination [11]. HWT 52°C for 30 minutes could not eradicate Lxx, but it was sufficient to reduce the incidence and to use for nursery standard procedure[13].

4. Conclusion
HWT 50°C for 2 h affected germination capability causing delayed and killed some of eye buds. However dipping setts in running water for 5 h before HWT 50°C for 2 h gave better germination, growth performance including production of tillers. HWT 50°C for 2 h significantly reduced RSD incidence, but it did not eliminate Lxx completely. HWT 45°C for 30 minutes and the use of bactericide was not able to reduce the incidence of RSD. Soaking RSD infected setts in running water could increase the incidence of RSD. Incidence of RSD was negatively correlated to plant height and internode length.

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