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Assessment of Rubber Clonal Rootstocks for the Tolerance of White Root Disease (*Rigidoporus microporus*) in Southern Thailand

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Abstract

Screening of rubber rootstocks that prevail good preference of plant growth with tentative tolerance of white root disease was done in southern Thailand. Seven selected clonal rootstocks and the major cultivated clone: RRIM 600 were grown in rhizoboxes (1 plant/1 rhizobox). An experiment was designed as an 8×2 factorial laid out in completely randomized design with the treatments comprised of 8 clones and 2 treatments of control and inoculation in 3 replicates. The experimental period was 24 weeks, the data of root distributions, growth, physiological responses and symptoms of rubber seedlings were recorded at 2-week intervals. After 24 weeks, the controlled clone EIRpsu 5 exhibited the highest shoot growth compared with the inoculum treatment clones. Root length density was accessed by scanning the root profile of each seedling from the panel of the rhizobox, it was found that the control treatment of the EIRpsu 1, EIRpsu 3, EIRpsu 4 and EIRpark clones had a high portion of root proliferation at 20 - 40 cm deep from the soil surface. The clone EIRpsu 5 exhibited the highest average root length density and the inoculum treatments of the EIRpsu 1, EIRpsu 2, EIRpsu 3 and RRIM 600 clones had high extension root growth at 0 - 20 cm deep. To investigate the physiological responses, the clone EIRpsu 5 showed the highest efficiency of photosynthetic rate with high stomatal conductance. According to the assessment of the disease index, it was found that the clone EIRpsu 4 exhibited the highest susceptibility score (54.09 %). Whereas, the lowest score was found in the clone EIRpsu 5 (12.12 %). This indicated that the clone EIRpsu 5 tended to be tolerant of white root disease with good performance of plant growth.

Keywords: *Rigidoporus microporus*, rootstock, physiological responses, disease index, white root disease

Introduction

Rubber trees (*Hevea brasiliensis* Muell. Arg.) are an important economic crop of Thailand. Nowadays, variability of climate change has been recognized as an important factor for root disease breakout [1]. One of the most serious concerns with such a serious impact is an increase in extreme incidents such as brown root disease, red root disease and white root disease. Particularly, white root disease caused by *Rigidoporus microporus* is a very important disease in rubber plantation and considered as the most destructive pathogen causing below and above ground symptoms as found in rubber trees worldwide [2]. It causes economic deprivation not only by loss of production, but also the persistence on dead or live root debris for a long time [3]. It forms many white, flattened mycelia strands which grow and extend rapidly through the soil in the absence of any woody substrate [4-6]. In addition, it was also reported that fruit trees were destroyed by this pathogen [7,8]. Like other white rot fungi that secrete a wide range of hydrolytic and oxidative enzymes [9], it is capable of degrading wood actively. Recently, white root disease has also been reported as a major cause of tree mortality in forest plantations of southern Thailand [10]. It was found that RRIM 600 is sensitive to white root disease [11] and there is no available resistant clone of rubber [12]. Thus, some early introduced rubber clones in southern Thailand were collected for rootstock screening for white root disease resistance. It was found that there were some clones that exhibited tentative tolerance to white root disease. Therefore, the objective of this study were: (i) to assess the effect of white root disease after inoculation on shoots, root growth and physiological responses of the rubber clones and (ii) to assess the survival of selected rubber clones for using as rootstocks.

Materials and methods

Plant material

The experiment was carried out in the glasshouses of the Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Seeds were collected from each rubber tree defined by its location by GPS (**Table 1**).

Table 1 Locations of sampling clones collected in Songkhla, Trang and Nakhon Si Thammarat provinces, Thailand.

Clone	Geographic coordinate	Places of collection
EIRpsu 1	7° 0' 31.7" N 100° 29' 40.3"	Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla. (PSU)
EIRpsu 2	7° 0' 23.1" N 100° 29' 52.8" E	Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Songkhla. (PSU)
EIRpsu 3	7° 0' 31.7" N 7° 0' 31.7" N	Drug store, Prince of Songkla University, Hat Yai, Songkhla. (PSU)
EIRpsu 4	7° 0' 29.8" N 100° 29' 58.6" E	Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla. (PSU)
EIRpsu 5	7° 0' 29.6" N 100° 30' 2.2" E	Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla. (PSU)
EIRpark	7° 0' 6" N 100° 27' 24" E	Hat Yai central park, Hat Yai, Songkhla.
EIRrak	7° 32' 33.1' N 99° 34' 35.6" E	Bangrak, Rubber plantation, Trang.
RRIM 600	8°15'42" N 99° 35'42" E	Nabon, Rubber plantation, Nakhon Si Thammarat.

Experimental design and treatment

The experiment was arranged as a completely randomized design using 8 rubber clones (EIRpsu 1, EIRpsu 2, EIRpsu 3, EIRpsu 4, EIRpsu 5, EIRpark, EIRrak and RRIM 600) with the 2 treatments of control and inoculation with 3 replications. All of the clones were grown in rhizoboxes (1 plant/1 rhizobox). Each rhizobox was made of a storm water pipe, 30.48 cm in diameter and 119.38 cm in length. Then, it was cut in half along the length of the pipe and covered with a transparent acrylic plate along the length as a panel of the rhizobox.

Pathogen

Fungal culture and growth conditions:

The fungus mycelium of white root disease was supplied by the Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Songkhla, Thailand. The white root rot isolated were collected in potato dextrose agar medium (PDA) for 4 - 5 days drill with a 5 mm cork borer in PDA, and incubated at room temperature (28±2 °C) for 4 - 5 days. Pieces of wood (size 2.54×5.08 cm) were placed on a culture medium for 5 days or until the fungus mycelium spread over the whole of the wood pieces.

Pathogenicity test:

Rubber seeds were germinated in sand for one month, then each seedling was transplanted to the rhizobox. The growth medium in each rhizobox contained mixed soil: manure: husk (3: 2: 2 ratio). Each 1-month seedling was inoculated by pre-infected rubber wood fragments (2.54×5.08 cm) applied against the taproot at 10 cm soil depth. Then, the seedlings were watered daily. Assessment of the severity of the white root rot disease was based on the disease index method devised by Kaewchai and Soytong [5]. Data collection as disease index (DI) was recorded at 120 days after starting the treatments. Symptoms of white root were observed at 2-week intervals. The disease index was categorized as follows: level 1 = healthy with green leaves, level 2 = 1 - 25 % yellow leaves, level 3 = 26 - 50 % yellow leaves, level 4 = 51 - 75 % yellow leaves and level 5 = 76 - 100 % yellow leaves. Infected root colonization was observed and recorded.

$$\text{Disease index (DI)} = \frac{(0 \times a) + (1 \times b) + (2 \times c)}{a + b + c} \times \frac{100}{x} \tag{1}$$

where 0,1 and 2 are infection categories
a, b and c are plants that fall into the infection categories
x is the maximum disease category which is 3

Growth of the rubber tree

Height: plant height was measured at 10 cm from the soil level to the top of the plant shoot with a tape measure.

Diameter: plant trunk diameter was measured at 10 cm from the soil level with a slide caliper.

Number of leaves: determined by counting the number of leaves per plant.

Photosynthetic rate and stomata conductance

The rate of photosynthesis and stomatal conductance of each seedling were measured during 10.00 - 12.00 a.m. by a portable photosynthesis system model Li-6400 (LCi Ultra Complex Photosynthesis System, USA). The measurements were made on 3 fully expanded leaves for each seedling. A least significant difference (LSD) test at $P < 0.05$ was employed for mean comparisons.

Root growth

The panel of each rhizobox was made of clear acrylic and covered with a black plastic sheet to avoid light exposure. To investigate root distribution, root images were scanned from the panel in 20 cm intervals by a scanner (Epson Perfection V330 Photo, Seiko Epson Corp., Japan.). The total length of the sample roots was determined by using Image Rootfly Software which is a free, open-source software application to aid researchers in rhizobox image analysis by GNU General Public License. Length, diameter, and color of roots as well as the alive and death rates were recorded. All the experimental data were stored in a single RFY file.

Statistical analysis

Analysis of variance (ANOVA) of data obtained was performed using R Gui software (version 2.12.0). Duncan's new multiple range test (DMRT) at $P \leq 0.05$ was employed for mean comparison.

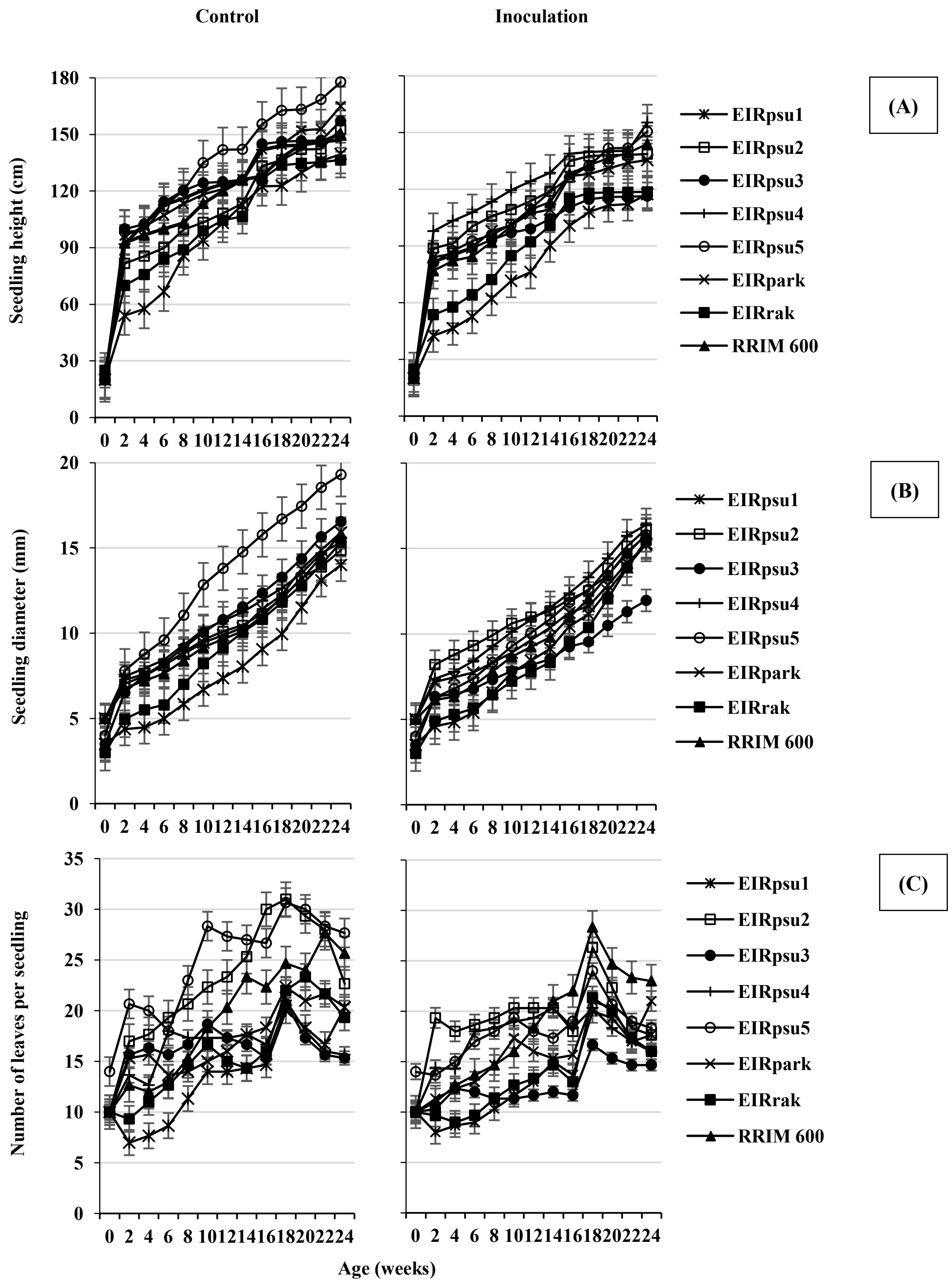

Figure 1 Shoot growth of the 8 clones in the control and inoculated rubber seedlings during the experimental period (A) increment of plant height, (B) increment of diameter, (C) increment of leaf number (error bars indicate mean ± SE).

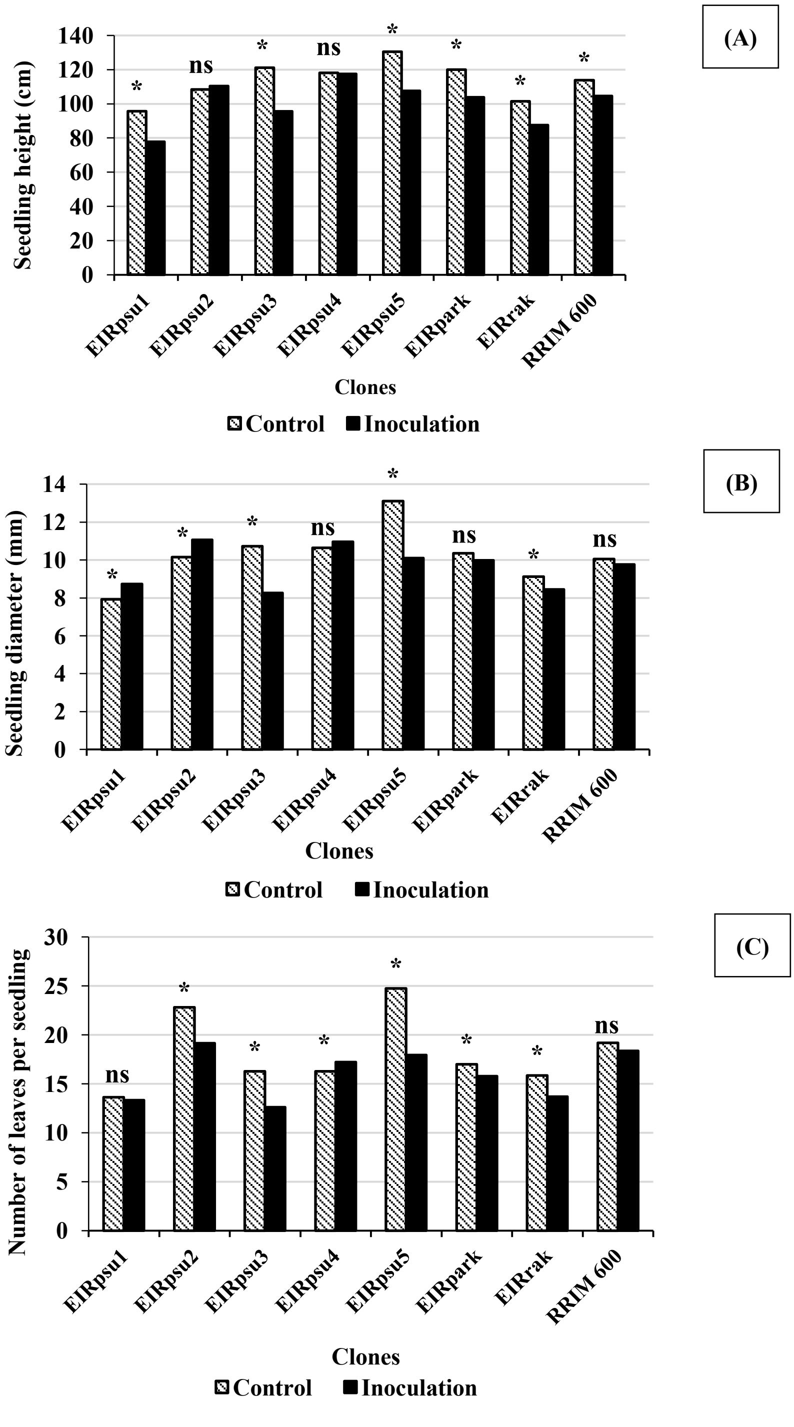

Figure 2 Comparison of average shoot growth of the 8 clones (A) increment of plant height, (B) increment of diameter, (C) increment of leaf number in the control and inoculated rubber seedlings.
* = significant difference between the control and inoculation of each clone at $P \leq 0.05$ by least significant difference (LSD) test.
ns = non significant difference between the control and inoculation of each clone.

Results and discussion

Shoot growth

A difference in shoot growth among the 8 rubber clones was found (**Figure 1**). There was also a significant difference in the plant height, leaf number and diameter. Comparing shoot growth response between the control and inoculum treatments, it was shown that the shoot growth of the control treatment was higher than the inoculum treatment. In the control treatment, the clone EIRpsu 5 had the highest plant height (130.45 cm), diameter (13.11 mm) and leaf numbers per plant (24.74 leaves per plant), which was significantly different from the other clones. Meanwhile, it was found that the clone EIRpsu 1 had low plant height, diameter and leaf number as shown in **Figure 1**. The inoculated EIRpsu 3 clone showed a sensitive response to the white root disease with a decrease in the diameter and leaf number. It was found that the pathogen affected shoot growth and plant responses differently among the clones. Comparing shoot growth response between the control and inoculation treatments, it was shown that the shoot growth of the control treatment was higher than the inoculum treatment. In addition, it was found that in the inoculation treatment, the clone EIRpsu 2 had the highest plant height, with the clones EIRpsu 1, EIRpsu 2, EIRpsu 4 having a high diameter. High leaf numbers were found only for clone EIRpsu 4 (**Figure 2**).

Root growth

Root growth of the rubber seedling was assessed at 20 cm interval depths. It was found that in the control treatment of EIRpsu 1, EIRpsu 3, EIRpsu 4 and EIRpark, the plants exhibited a high portion of root proliferation in the 20 - 40 cm layer from the soil surface. In contrast, the plants in the inoculum treatment of EIRpsu 1, EIRpsu 2, EIRpsu 3 and RRIM 600 exhibited high extension root growth at 0 - 20 cm (**Figure 3**). The clone EIRpsu 5 exhibited the highest total average root length density compared with the other clones. While the lowest root length density of clone RRIM 600 was found at various depths. All clones of the control treatment were better in root growth compared with the inoculated treatment (**Figure 4**).

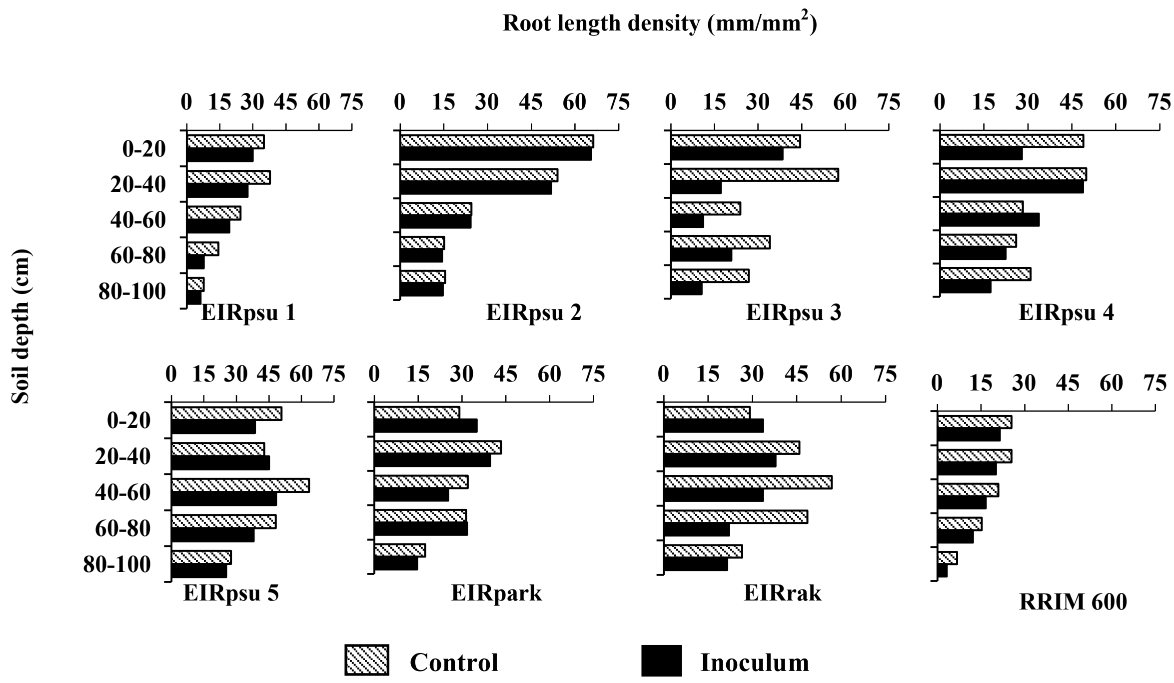

Figure 3 Comparison of the root profile between the control and inoculum treatments of the 8 clones.

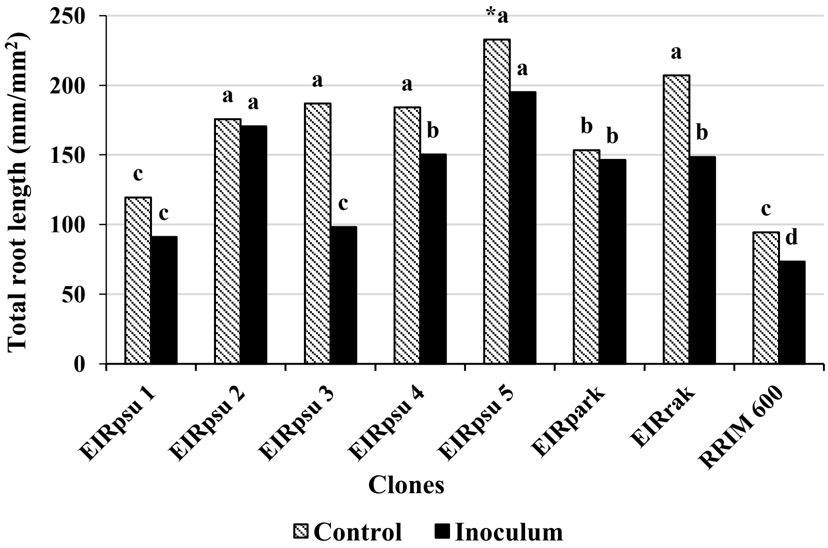

Figure 4 Comparison of the average total root length density (mm.mm^{-2}) among the 8 clones in the soil profile observed from the rhizobox panel in the control ( ) and inoculation ( ) treatments.
*Different letters on each column indicate highly significant difference ($P \leq 0.01$) by Duncan's new multiple range test.

Physiological responses

Figure 5 shows the control treatment of EIRpsu 5 clone exhibited the highest average photosynthetic rate (7.38 µmol/m^2/s), and stomatal conductance (293.33 mmol/m^2/s). While the clones EIRpsu 1 had the lowest photosynthetic rate (6.52 µmol/m^2/s) and clone EIRrak had low stomatal conductance followed by other clones. In the inoculated treatment, it showed that the clone EIRpsu 5 exhibited the highest average photosynthetic rate (7.22 µmol/m^2/s), and stomatal conductance (289.58 mmol/m^2/s) and the clones of EIRpsu 4 had the lowest photosynthetic rate (6.31 µmol/m^2/s) and stomatal conductance (219.58 mmol/m^2/s). The photosynthetic rate and the stomatal conductance of each rubber clone was in the range 4.52 - 11.92 mmol/m^2/s and 110 - 440 mmol/m^2/s, respectively. Comparing photosynthetic response of the control and inoculated treatments, it was found that the rubber clones in the control treatment tended to exhibit a higher average of photosynthetic rate and stomatal conductance than the inoculated treatment. The lowest average photosynthetic rate and stomatal conductance was found for EIRpsu 1 clone in the control treatment. However, there were significant differences in the photosynthetic rates in the control and inoculum treatment of EIRpsu 1, EIRpsu 3, EIRpsu 4 and RRIM 600 clones. However, it was not significantly different in the stomatal conductance of EIRpsu 1, EIRpsu 5 and EIRpark clones (**Figure 6**).

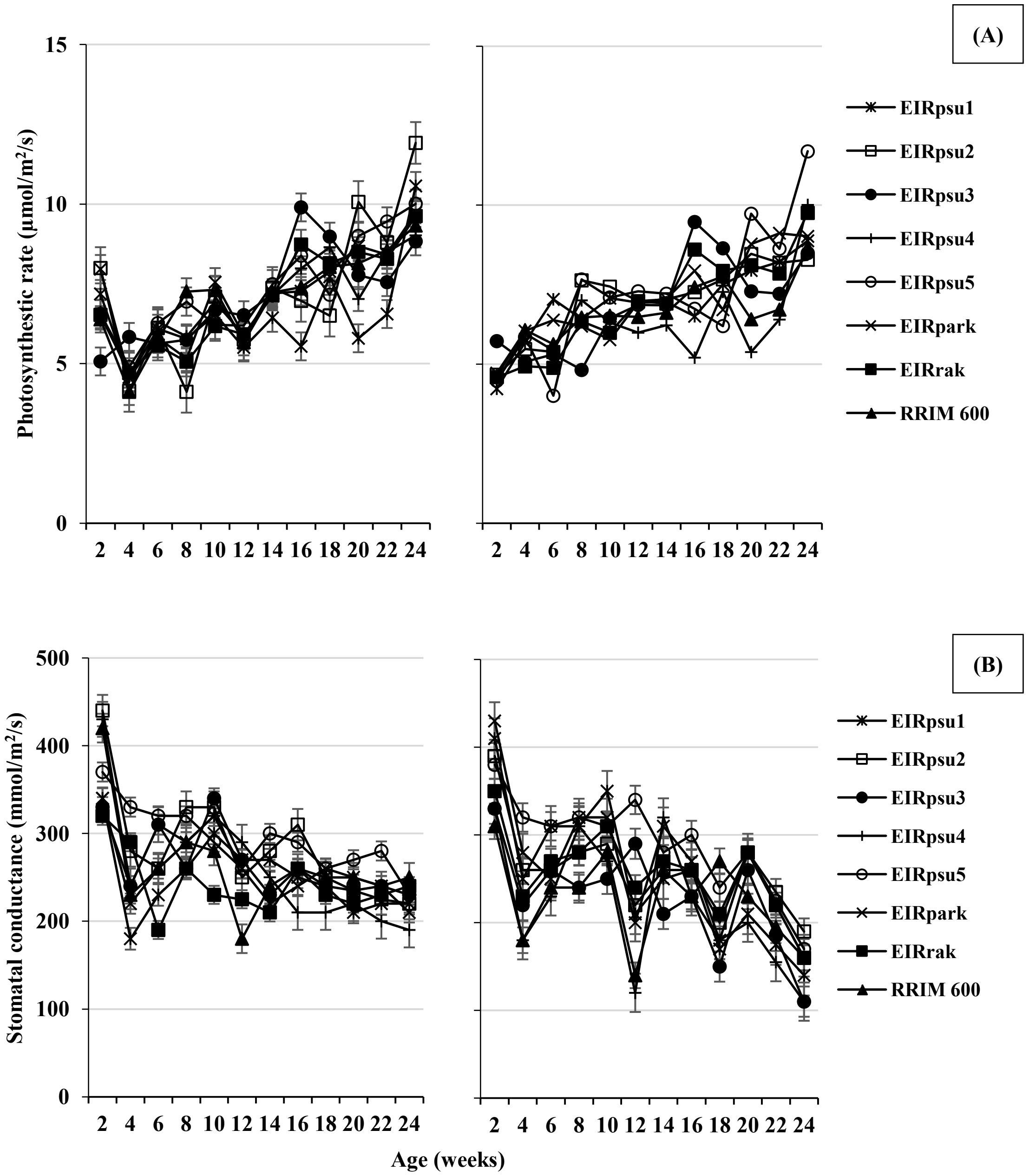

Figure 5 Physiological responses of the 8 clones in the control and inoculated rubber seedlings during the experimental period (A) photosynthetic rate, (B) stomatal conductance at 24 weeks.
(error bars indicate mean ± SE).

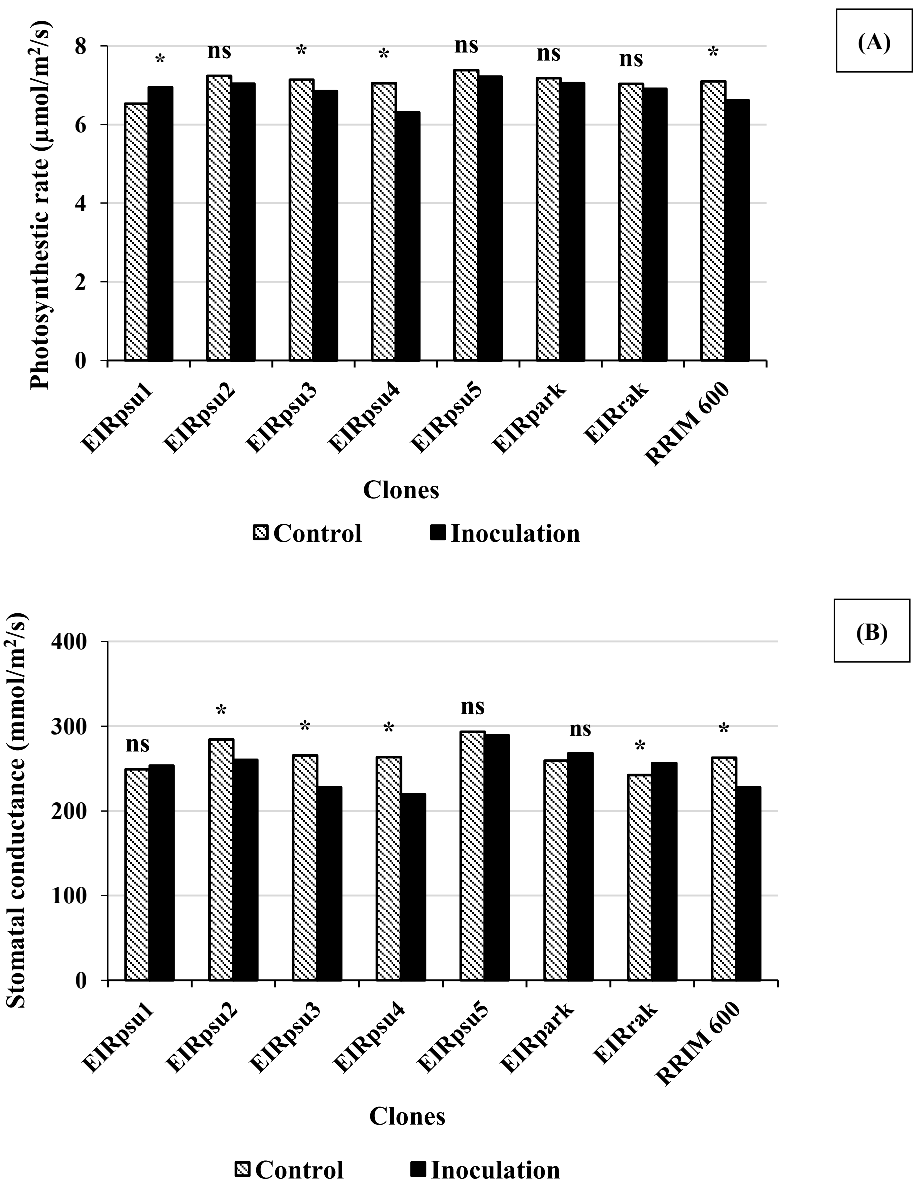

Figure 6 Comparison of average physiological responses (A) photosynthetic rate (µmol/m^2/s), and (B) stomatal conductance (mmol/m^2/s) at 24 weeks of the all clones for both the control and inoculation treatments.
* = significant difference between the control and inoculation treatments of each clone at $P \leq 0.05$ by least significant difference (LSD) test.
ns = non-significant difference between the control and inoculation treatments of each clone.

Symptoms of the white root disease

The effects of white root disease on the 7 selected clones and RRIM 600 clone at 24 weeks are shown in **Table 2**. It was found that each clone exhibited different response to the white root disease. The clones of EIRpsu 3 (49.49 %), EIRpsu 4 (54.09 %), EIRrak (41.16 %) and RRIM 600 (51.76 %) had high impact from the white root disease and chlorosis was observed in the leaves with falling-off at 24 weeks after inoculation. In addition, it was found that EIRpsu 1, EIRpsu 2, EIRpsu 5 and EIRpark clones showed fewer symptoms of the white root disease compared with the other clones. The disease index showed that the clone EIRpsu 4 (54.09 %) had the highest susceptibility score, whereas the lowest score was found in the clone EIRpsu 5 (12.12 %).

Table 2 Disease index (DI) after inoculated rubber clone seedling at 24 weeks with *R. microporus*.

Clone	% Survival	% DI
EIRpsu 1	100	36.36
EIRpsu 2	100	30.30
EIRpsu 3	33.33	49.49
EIRpsu 4	0	54.09
EIRpsu 5	100	12.12
EIRpark	100	27.27
EIRrak	66.66	41.16
RRIM 600	33.33	51.76

Growth responses

The fungus attacked the rubber roots causing a decrease in shoot growth. It was evident that the shoot growths of the control clones were higher than the inoculated clones. The results of the control treatment showed that the height, diameter and leaf number per plant continuously increased. In the control treatment, the clone EIRpsu 5 had the highest height (130.45 cm), diameter (13.11 mm) and leaf number per plant (24.74 leaves per plant) and the highest root length density was found in clone EIRpsu 5. Russell [13] reported that shoot and root growth were related to environmental conditions and changing environmental conditions could affect the dry weight of roots and trees. Thus, analyzing the growth is determined using the principles of the relationship between the source and sink. Meanwhile, it was found that the inoculated clone EIRpsu 4 had a plant height of 117.64 cm. The EIRpsu 2 clone had the highest diameter (11.06 mm) and leaf number (19.15 leaves per plant) and it was significantly different from the other clones. Zaini and Halimoon [14] reported that the changes are influenced by many factors and the major factors are water content, water tension inside the rubber plants and the fungal attack to the plant root causes a loss of their function to uptake water and nutrients from soil. The fungus harmed the life span of the plants. This might be due to the impact of *R. microporus* on root growth leading to limitation of the water uptake. Nahar and Gretzmacher [15] also reported that plant growth was limited because of increasing stress.

Root responses

According to the root profile study of the control treatment, it indicated that the clones EIRpsu 1, EIRpsu 3, EIRpsu 4 and EIRpark exhibited high root proliferation at a depth of 20 - 40 cm. The inoculum treatment, showed that clones of EIRpsu 1, EIRpsu 2, EIRpsu 3 and RRIM 600 had high extension root growth in shallow 0 - 20 cm layer. The clone EIRpsu 5 exhibited the highest total average root length density compared with the other clones. While the lowest root length density was found for the clone RRIM 600. The resulting similarity with Cherngchalard [16] reported that the rubber seedling grown in a minirhizotron had high root proliferation at 20 - 40 cm from the soil surface. The root proliferation of the seedlings of clone RRIM 600 and GT 1 were located within 0 - 15 cm and 20 - 40 cm from the soil surface, respectively. Liedgens *et al*. [17] reported that the pattern of root distribution is also affected by many factors. Hamblin [18] suggested that root development in plants is governed by various factors such as nutrient availability, soil physical properties and genetic characters.

Physiological responses

The investigation of physiological parameters can support the plant response to the white root disease because photosynthetic rate, and stomatal conductance of the inoculated plant decreased. The plants in the control treatment showed high physiological responses. According to Lee and Noraini, [19]; Zaini and Halimoon [14], studied the ability of *Catharanthus roseus* stem extract to control white root rot disease of rubber trees, it was found that the fungus will start to attack the plant root at 2 weeks after the inoculum was planted and it depends on the plants as every plant has its own defense to the disease. Since the *R. microporus* cannot produce their own food and they need to rely on their host (rubber plants) when the leaves are old or there is damage on the leaves, it might turn yellow or orange [14]. The chlorophyll was translocated out of the leaves and appeared yellow before death. Zaini and Halimoon [14] reported that chlorophyll contents in the leaves also influence the leaves performance. When the level of chlorophyll decreases, the leaves turn chlorosis leading to high leaf falling rates. Sudden changes in temperature can lead to leaves turning yellow or brown and thus cause the leaves to drop. The reductions on stomata conductance of guard cells force the stomata to close, so that it will reduce the transpiration rate and photosynthesis rate [13,20]. Somjun [21] also supported physiological response of the rubber that it could be assessed by the stomatal conductance. It was found that there was a bit of a difference in the stomatal conductance and photosynthesis rate among the clones. Kröber *et al*. [22] reported that stomatal conductance and stomatal regulation were found to be related to morphological, anatomical and chemical leaf traits. Supacharoenkun [23] reported that the difference in photosynthesis rate and stomatal conductance also depend on the different clones. The photosynthetic rate of the leaves decreased as the leaf water potential decreased or during water stress [24].

Symptom development

White root disease, *R. microporus* could infect all stages of the plant from the seedling. The clone of EIRpsu 4 showed yellowing leaves on one or a few branches at 12 weeks and the symptoms included wilting and yellowing of the leaves, defoliation and white mycelium on the root system after infection. Finally, the tree dies. The clone of EIRpsu 1, EIRpsu 2, EIRpsu 5, and EIRpark exhibited high survival and lowest susceptibility score until the end of the observation. The clones EIRpsu 3, EIRrak and RRIM 600 were affected by the white root disease and the leaves were chlorosis with falling after 12 weeks. This results was similar to the report of Kaewchai and Soytong [5] who found that RRIM 600 showed symptoms of yellowing leaves at 70 days and the roots of the dead tree was covered with the rhizomorph of the pathogen and it produced fruiting bodies at the collar of the dead stem. Wattanasilakorn *et al*. [10] studied screening of rubber rootstocks for the white root disease resistance and compared with RRIM 600 and GT 1, it was found that GT 1 seedlings were sensitive to the white root disease.

The symptom development of the white root disease also depends on many environmental factors. Most commonly, the symptoms would start after the infection with *R. microporus*, it appeared to exhibit almost similar foliar symptoms. Progress of the disease was generally observed first, as yellowing followed by wilting, defoliation and finally death of the host. In addition, progress of these symptoms was similar to the report by Mohd *et al.* [25,26], and roots of saplings inoculated with *R. microporus* had white rhizomorphs on their surface. Nissapa and Chuenchit [27] reported that RRIM 600 and BPM 24 clones were highly susceptible to white root disease. In this study, the result also showed that the RRIM 600 clone was sensitive to the white root disease. Khonglao [28] reported that most rootstocks from the seed of early introduced clones to Thailand, and it is suggested that rubber clones that are tolerant of white root disease are needed to be selected from early introduced clone population.

The presence of the rubber clones showed that the plants have the ability to adapt after infection as shown by the positive result of shoot growth, (height, diameter and leaf number), root growth, and physiological response.

Conclusions

It was found that the shoot growth, root development and physiological responses of the control plants were higher than the inoculated plants. The clone EIRpsu 5 exhibited the highest shoot growth and with the highest average root length density. The physiological responses of the clone EIRpsu 5 showed the highest efficiency of photosynthetic rate with high stomatal conductance. According to the assessment of the symptom development of the white root disease, it showed that clone EIRpsu 4 exhibited the highest susceptibility score, whereas the lowest score was found in the clone EIRpsu 5. This suggested that the clone EIRpsu 5 tended to be tolerant of the white root disease with good performance in plant growth.

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