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Screening of rubber (*Hevea brasiliensis* Muell. Arg.) rootstocks for the white root disease resistance

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The white root disease caused by *Rigidoporus microporus* (Sw.) Overeem is a destructive disease in rubber plantation, particularly in southern Thailand. It persists on dead or live root debris for a long time. In this study, resistant clones of white root disease were screened. Pathagenicity tests of *R.microporus* in 10 local clones (PSU1, PSU2), Kantang, Bangmark, Sakraphangsurin, Bangrak, Khaowiset, Wangkere, Bangdee and Huaiyot districts(were done, compared with clone RRIM 600 and GT 1. The experiment was designed as a completely randomized design (CRD) with 5 replications. The following data were recorded for 2-week interval within 5 months: root distributions, area under disease progress curves (AUDPC), growth and symptom of rubber seedlings. Results indicated that the most of active root proliferation of 45-60 cm soil layer depth from the soil surface. Root growth of seedlings from clone Bangmark and Huaiyot districts showed significantly higher than RRIM 600, GT 1 and the other clones. With the AUDPC observation, the seedlings from clone Bangmark, Kantang and Prince of Songkla University (PSU1) were significanly higher P>0.05 (AUDPC) than the other clones. Growth of each clone was monitored by measuring height, circumference and number of petiole per seedling, the seedlings from clone Bangrak exhibited the highest growth. Symptom development of the seedlings from clone Kantang, Khaowiset districts and GT 1 clones were evident, around 50%. Among 10 local clones, RRIM 600 and GT 1 clones, the seedlings from Sakraphangsurin, Bangrak districts, PSU1 and PSU2 clones tended to exhibit white root disease resistance.

Key words: *Rigidoporus microporus*, area under disease progress curves, white root disease, tolerance, local clone

Introduction

White root disease of rubber trees caused by *Rigidoporus microporus* Sw. (Overeem is well known as a destructive disease in rubber plantation in

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many countries: Cameroon, Ivory Coast, Ghana, Nigeria, Gabon, India, Indonesia, Malaysia, Sri Lanka, Thailand, West and Central Africa (Hashim and Malik, 2006; Jayasuriya and Thennakoon, 2007; Kaewchai *et al*., 2010). The disease causes economic lost not only for the lost of production, it also persists on dead or live root debris for a long time. It forms many white, flattened mycelial strands which grows and extends rapidly through the soil in the absence of any woody substrate (Nandris *et al*., 1987; Kaewchai and Soytong, 2010). The root of healthy rubber tree can be infected by contact with disease source, such as rhizomorphs, infected root, dead stump, or wood debris (Nandris *et al*., 1987; Guyot and Flori, 2002; Kaewchai *et al*., 2010). It can result in substantial death of trees and sometimes losses of a whole stand (Guyot and Flori, 2002). The fruiting bodies of this fungus form at the collar of the dead stem which produce a large number of basidiospores (Figure 1), but it has a limited role in dissemination of this disease (Nandris *et al*., 1987). Preliminary study reported that the seedling of RRIM 600 mainly grown in Thailand is sensitive to the white-root disease. (Holiday 1980) reported that there is no resistant clone of rubber available. Hence, the objective of this study was to assess white root disease AUDPC, symptom development, root growth and growth of rubber trees with screening of tentative resistance clones

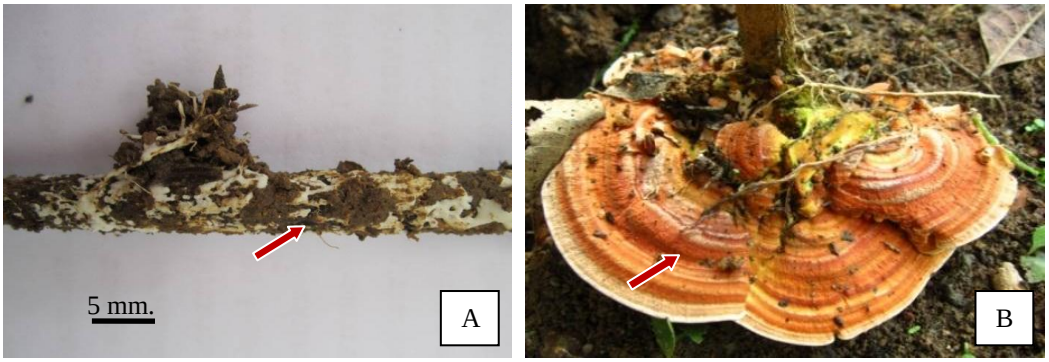

Fig. 1. Rhizomorph at the root (A) and fruiting body at the collar of dead stem of *R. microporus* (B)

Materials and methods

The study was carried out from February to September 2011, in the glasshouse of Faculty of Natural Resources, Prince of Songkla University, Songkhla Province, Thailand. Root growth of 10 local clones collected from different areas in Songkhla, Trang and Suratthani province were tested (Table 1). Clone RRIM 600 and GT 1 were used for comparison.

Table 1. Collected locations of local and cultivated clones

Name	Local Districts	Places of collection
Clone#1	Hat Yai (PSU1)	Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla
Clone#2	Hat Yai (PSU2)	Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Songkhla
Clone#3	(Kantang)	Kantang, Rubber plantation, Trang
Clone#4	(Bangmark)	Bangmark, Rubber plantation, Trang
Clone#5	(Sakraphangsurin)	Sakraphangsurin, Rubber plantation, Trang
Clone#6	(Bangrak)	Bangrak, Rubber plantation, Trang
Clone#7	(Khaowiset)	Khaowiset, Rubber plantation, Trang
Clone#8	(Wangkere)	Wangkere, Rubber plantation, Trang
Clone#9	(Bangdee)	Bangdee, Rubber plantation, Trang
Clone#10	(Huaiyot)	Huaiyot, Rubber plantation, Trang
GT 1	(Surat Thani)	Rubber Research Institute of Surat Thani, Suratthani
RRIM 600	(Klonghoykhon)	Klonghoykhong, Rubber plantation, Songkhla

The experiment was designed as a completely randomized design (CRD) with five replicates. Each clone of the 5-month rubber was planted in a rhizobox Figure (2) 30.48x119.38 cm (contained mixed soil) soil: manure: husk; 3: 2: 2, then the soil preparation analysed at Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Songkhla. Then, the rhizobox were lined with The rhizobox were lined with spacing 1m x1m. Root growth of the rubber seedling was assessed in each 15 cm-interval depth. The panel of rhizobox was made of clear acrylic and covered with the black plastic sheet to avoid light exposure. To investigate root distribution, a transparent plastic sheet (30.48 x 119.38 cm.) was lined on the panel. Consequently, the roots were traced using a permanent marker pen with different colors along observation dates. The total length of the sampled roots was measured by using Image Rootfly Software a free, open-source software application to aid researchers in minirhizotron image analysis by the 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.

Fig. 2. The rhizobox used for root investigation

Pathogenicity test

Rigidoporus microporus was isolated from infected roots of rubber trees by tissue transplanting technique. The culture was maintained in potato dextrose agar (PDA) medium and deposited at the Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Songkhla, Thailand. Pathogenicity tests of *R. microporus* were done in 10 local clones, RRIM 600 and GT 1 clones. Each treatment consisted of one healthy and inoculums which placed into the rhizobox closing to the root system. Symptoms of white root were observed at 2-week interval. Evaluation of disease infection was conducted according to Kaewchai *et al*. (2010) with the following modification: The Disease Index)DI (were determined as follows: level 0 = healthy, green leaves, level 1 = 1-25 %yellow of foliage, level 2 = 26-50 %wilting, level 3 = 51-75% defoliation and level 4 = 76-100% death of plant. And evaluation the distribution of the white root disease from the soil surface was also observed up to 16 weeks by using the following modification:

Level 0 = not found in the root fungal infections.
Level 1 = fungal infections in the root is less than 1%
Level 2 = fungal infections in the root, 1-10%
Level 3 = fungal infections in the root, 11-50%
Level 4 = fungal infection in the root, 51-90%
Level 5 = fungal infections in the root of more than 90%

AUDPC analysis

Data for distribution of the white root disease in root rubber were assessed from different soil layer depths. Thus, the derived disease parameter, and the area under disease progress curve (AUDPC) were calculated according to the equation of Campbell and Madden (1991) using the following formula:

$$AUDPC = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

where n = the total number of observations

Y_i = disease severity in percentages at the i^{th} observation

t = time in days after white root disease inoculation at i^{th} observation

$t_{i+1} - t_i$ = interval between two consecutive observations

Analysis of disease development were performed when greater quantification that needed for resistance evaluation. The disease progress curve represented an integration of all host, pathogen and environmental effects occurring during disease development

Means were compared with the Duncan multiple range test (DMRT). These disease severity were recorded at week 2, 4, 6, 8, 10, 12, 14 and 16, respectively.

Results and discussions

Soil properties

The texture of the soil was determined along with pH, organic matter, macro and micro nutrients (Table 2). The soil was sandy clay loam in texture with moderate compaction. It was characterized as low pH (5.94) and contained reasonable amount of organic carbon, organic matter and total nitrogen. Exchangeable cations were generally low with Ca (3.17 cmol/kg) being the highest, Mg was recessive in the cation exchange (1.11 cmol/kg) but high available phosphorus and potassium.

Table 2. Analysis of soil used in the experiment

Soil properties	characteristics
pH	5.94
Organic. C (%)	1.54
Organic matter	2.65
Nitrogen (%)	0.13
Available P (mg/kg)	61.88
Available K (mg/kg)	911.00
Exch. Ca (cmol/kg)	3.17
Exch. Mg (cmol/kg)	1.11

Growth of the rubber tree

The result of growth 10 local clones, RRIM 600 and GT 1 clones is shown in Table 3. Plant height, number of petiole per seedlings and circumference at 15 cm above soil were measured, it indicated that there were significantly different away the clones. The local clone from Bangrak, increased from 68.460 to 105.117 cm, and its circumference increased from 9.070 to 11.220 cm. While RRIM 600 clone had the average number of petioles per seedling 21.514. In addition, comparing between clone RRIM 600 and GT 1, it was found that RRIM 600 had better growth than the GT 1. Comparing among the 10 local clones, it showed that PSU2 clone had the highest 19.943 petioles per seedling. (Table 3)

Table 3. The average height, girth and number of petiole per seedlings of 12 rubber clones

Treatments	Mean		
	Height (cm)	Circumference (cm)	No. of petiole per seedling
Clone#1	77.654 bc	7.945 c	17.457 ab
Clone#2	86.831 ab	9.156 b	19.943 ab
Clone#3	72.663 bc	7.479 cde	19.286 ab
Clone#4	78.211 bc	7.461 cde	19.486 ab
Clone#5	86.497 ab	7.705 cd	18.314 ab
Clone#6	105.117 a	11.220 a	16.943 abc
Clone#7	76.800 bc	6.727 def	15.171 bc
Clone#8	94.903 ab	6.375 ef	17.514 ab
Clone#9	40.706 d	4.865 g	12.086 c
Clone#10	72.551 bc	7.481 cde	17.514 ab
GT 1	55.229 cd	5.799 fg	16.543 abc
RRIM 600	86.563 ab	8.328 bc	21.514 a
F-test	*	*	*
C.V. (%)	28.431	9.359	26.885

Means with the same superscript in each column are not significantly different by LSD 0.05
* significant difference at $P \leq 0.05$ by Duncan Multiple Range Test

Root growth of the rubber tree

Although the root length density in the surface layer manifested different treatment, there was no definite pattern related to placement. Root distribution and root development of local rubber clones were compared. The root in the rhizobox indicated that most of active roots that located within 45-60 cm depth from the soil surface and rubber roots were proliferated rapidly. In addition, root growth of seedlings from clone Bangmark and Huaiyot showed significantly higher than those of the other clones. Active roots of GT 1 were located within 75-90 cm depth from the soil surface. Root growth pattern of RRIM 600 were intensed at the 0-15 cm. depth. While root distribution decreased in the 30-45 cm layer. This indicated that rubber root freedom was at surface with 55% of the root activity confining to the top 10 cm of soil layer. Root activity declined with increasing depths (George *et al*., 2009). Nares and Sayan (2551) evaluated the growth of rubber tree roots by using a minirhizotron, and it was found that the high root density was at the 0-10 cm. soil depth. However, Hamblin (1985) suggested that root development in any plant is governed by factors such as nutrient availability, soil physical properties and genetic characters. One problem of rhizobox observation is the overestimating root length density at depth, which may be due to roots channeled down the vertical tube to soil interface. The profile of root length density of the all clones were different as shown in Figure 3 and 4.

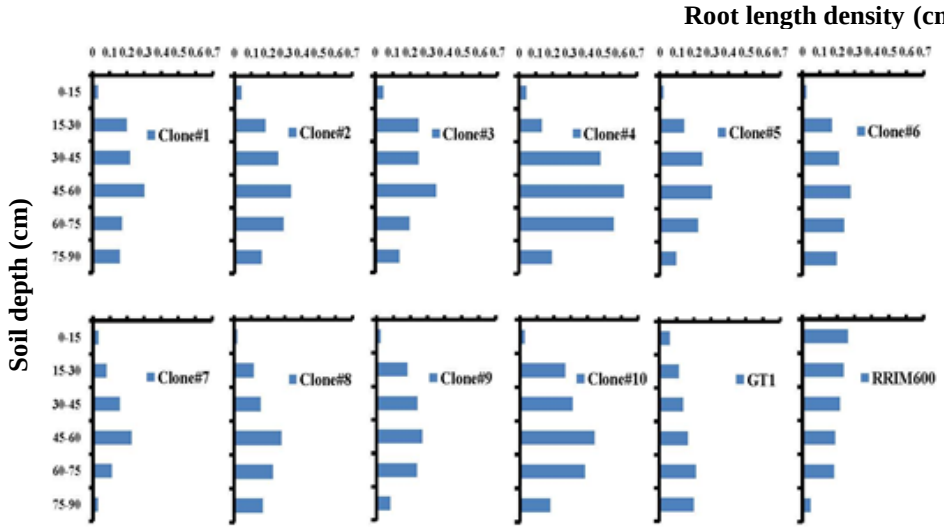

Fig. 3. Comparison of root profiles of the rubber seedlings among the all clones

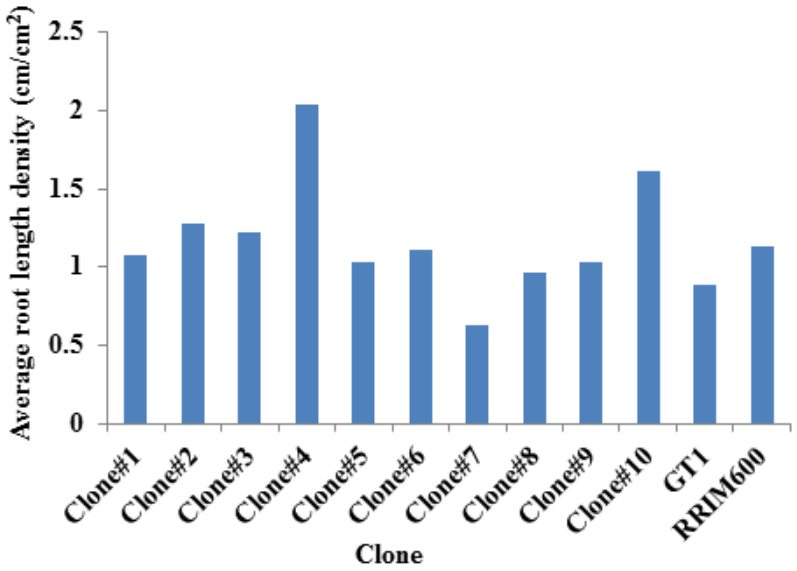

Fig. 4. Average root length density of each clone assessed from the panel of each rhizobox

Severity and AUDPC

Among 10 local clones, RRIM 600 and GT 1 clones, the development of symptoms caused by *R. microporus* showed that symptoms were different at various soil depths. The dorsal root was possessed with rhizomorph of the pathogen and it produced fruiting body at the collar of the dead stem. It also found that AUDPC is the one that may be used to distinguish clones of rubber to the destruction of the white root disease. Disease severity scores and AUDPC value provided evaluation of the reaction of the clones to 0-15, 15-30, 30-45, 45-60, 60-75 and 75-90 cm to *R. microporus*. The values of AUDPC were assessed during the entire period of 16 weeks as disease severity scores. (Table 3). The results showed that after inoculation with *R. microporus* at 2, 4, 6, 8, 10, 12, 14 and 16 weeks, the seedling of all clones disease severity expressed as AUDPC were significantly different ($P>0.05$) within 15-30 and 30-45 cm. depth of the soil surface. At soil depth of 30-45 cm, seedlings from Bangmark, Kantang and PSU1 clones were AUDPC values high as 32, 23.25 and 21.25. While Sakraphangsurin, Bangrak and Khaowiset clones had consistently lower AUDPC values of 8.25, 5.67 and 1.5 (Table 4) Joko (2009) reported that the development of the disease depends on many factors such as humidity, temperature, pH, soil porosity and soil characteristics. Besides, this experiment was a preliminary study in a short period, therefore, it needs to be investigated in long term.

Table 4. Area under disease progress curve)AUDPC(among the 12 clones at various soil layers (0-15, 15-30, 30-45, 45-60, 60-75, 75-90 cm.)

Name	AUDPC						Mean
	0-15	15-30	30-45	45-60	60-75	75-90	
Clone#1	0	13.75a	21.25ab	35.75	21.25	16	18
Clone#2	3.5	3.5bc	13.75bc	19.25	17.75	16.5	12.38
Clone#3	3.5	14.25a	23.25ab	20	8	4.25	12.21
Clone#4	0	0c	32a	23.75	24	28.75	18.08
Clone#5	1.5	7.5d	8.25bc	9.25	16	14.25	9.46
Clone#6	0	0c	5.67bc	13	3	1.5	3.86
Clone#7	1.5	1.5c	1.5c	25.25	21.25	17.5	11.42
Clone#8	0	11.75ab	17.75abc	19.75	15.5	10.25	12.5
Clone#9	0	0c	12bc	12	14.75	6	7.46
Clone#10	3.75	3.75bc	16.5abc	23.25	23.5	23.25	15.67
GT 1	3.75	3.75bc	16.5abc	16.25	10.75	9.25	10.04
RRIM 600	0	0 c	12.25bc	15.5	18	14	9.96
P=0.05	ns	*	*	ns	ns	ns	ns
CV(%)	295.27	111.26	64.86	59.19	85.57	82.10	74.34

* significant difference at P≤0.05 by Duncan Multiple Range Test
ns = non significant difference by Duncan multiple range test at P>0.05 probability level
AUDPC = Area under disease progress curve

Symptoms of the white root disease

According to the study of 10 local clones, RRIM 600 and GT 1 clones inoculated with *R.microporus*, the results showed that each clone had symptom development of the white root disease was different. Seedlings from Kantang, Khaowiset and GT 1 clones had affected the white root disease 50% (Table 5). In addition, it was found that PSU1, Sakraphangsurin, Bangrak and PSU2 clones showed symptom development of the white root less than all other clones, this indicated to immune for white root.

The symptom development the white root disease depends on the environment factors. Most commonly, the symptoms would start after the infection with the *R. microporus*, it appeared to exhibit almost similar foliar symptoms. Progress of 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 Farid *et al.*, (2001, 2006), and roots of saplings inoculated with *R. microporus* had white rhizomorphs on their surface.

Table 5. White root disease seedlings of 12 rubber clones

Name	No. of seedlings affected the white root disease/total seedlings	Score for the white root disease (16 week)
Clone#1	0 / 4	0
Clone#2	0 / 4	0
Clone#3	2 / 4	4
Clone#4	1 / 4	2
Clone#5	0 / 4	0
Clone#6	0 / 4	0
Clone#7	2 / 4	4
Clone#8	1 / 4	4
Clone#9	1 / 4	4
Clone#10	1 / 4	4
GT 1	2 / 4	4
RRIM 600	1 / 4	4

Conclusion

Among the seedlings 10 local clones, RRIM 600 and GT 1 clones, the white root and root growth were different. Most of the local clones, their of active roots of were located within 45-60 cm depth from the soil surface. Whereas the of root proliferation the seedlings of clone GT 1 and RRIM 600 were located within 75-90 cm and 0-15 cm depth from the soil surface, respectively. The growth of seedling from clone Bangrak was the most. The values of AUDPC and severity score assessment of the white root disease showed that GT 1 seedlings were sensitive to the white-root disease. While the seedlings from clone PSU1, Sakraphangsurin, Bangrak and PSU2 showed that they were tentative resistance to the white root disease more than the other clones.

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