Agriculture and Natural Resources 50 (2016) 98–103

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original article

Growth performance of clonal rubber rootstocks and combining ability test with the scion of clone RRIM 600

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ARTICLE INFO

Article history:
Received 24 December 2014
Accepted 29 July 2015
Available online 9 June 2016

Keywords:
Compatibility
Root length density
Rootstocks
Rubber
Scion

ABSTRACT

Past screening of rubber rootstocks in southern Thailand has revealed five clones that showed good plant growth with tentative tolerance to white root disease. The current study tested the combining ability of these clones with the scion of clone RRIM 600, commonly grown in Thailand. The five clonal rootstocks and the clone RRIM 600 were grown in rhizoboxes (10 × 45 × 100 cm) in a completely randomized design, consisting of six treatments each with four replicates, making a total of 24 rhizoboxes. After growing for 6 mth, each seedling (1 cm stem diameter) was bud grafted with the scion RRIM 600. Root growth of the rootstocks was assessed by scanning a glass panel in the side of each rhizobox after 3 mth. It was found that the seedlings of clone#5 exhibited significantly higher root growth with tentative high shoot growth compared with the remaining treatments. The results showed that clone#5 exhibited the highest average root length density at 1.26 cm/cm^2 followed by clone#2 (0.90 cm/cm^2) with a significant difference from the other clones. The investigation of graft union development showed that callus tissues developed on the graft union within 5 d after bud grafting (DAB). The graft union growth was almost fully developed over the wound areas and callus bridge formation was evident within 10 DAB. The new vascular tissues were connected with old cambium and vascular tissues of the scion and rootstocks within 20 DAB. The photosynthetic rate and stomatal conductance were assessed and showed 100% successful budding of rootstocks of clone RRIM 600 and clone#4, whereas the other clones achieved 75% success.

Introduction

Southern Thailand is the country's main area of rubber planting, clonal material production and rubber production with Thailand's area of rubber plantation and the export of Thailand in 2013 being 3,548,274 ha and 3,664,941 t, respectively (Rubber Research Institute of Thailand, 2014). Normally, rubber seeds are collected from the smallholder's rubber plantation and therefore, most seeds are from rubber clone RRIM 600, which is mainly grown in southern Thailand and is sensitive to white root disease (Wattanasilakorn et al., 2012). Kaewchai and Soytong (2010) also noted that white root disease is a severe epidemic in southern Thailand. Therefore, some early introduced rubber clones were collected for rootstock screening of rootstock white root disease resistance and it was found that there were some clones that exhibited tentative tolerance to white root disease (Wattanasilakorn et al., 2012). However, those rootstocks needed to be tested for their combining ability. Donald (1973) reported that 91% of the grafts were compatible when seedling families were grafted upon rootstocks which were compatible with both parents. During the formation of the graft union, researchers have observed callus proliferation (from both the rootstock and the scion), callus bridge formation, differentiation of new vascular tissue from callus cells and the production of secondary xylem and phloem (Hartmann et al., 2002). A low level or incorrect callus formation between the rootstock and scion could lead to defoliation, a reduction in scion growth and low survival of grafted plants (Oda et al., 2005; Johkan et al., 2009). The objectives of this study were: 1) to study the growth and development of the rubber clones used as rootstocks; 2) to study the compatibility of the rubber rootstocks with clone RRIM 600; and 3) to study the physiological responses of the RRIM 600 scion on the screening rootstocks.

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http://dx.doi.org/10.1016/j.anres.2015.07.003

Materials and methods

The study was carried out from April 2012 to August 2013 in a glasshouse of the Faculty of Natural Resources, Prince of Songkla University, Songkhla province, Thailand.

Plant materials

The root growth of five early introduced clones collected from different areas in Songkhla province was tested. Collected seeds from each tree were georeferenced using a global positioning system (Table 1). Clone RRIM 600 was used for comparison. The experiment was established as a completely randomized design with six treatments and four replicates (one plant per replication). The seedlings (aged 2 mth) were grown in rhizoboxes ($10 \times 45 \times 10$ cm). One seedling was grown in each rhizobox (Fig. 1).

Root growth

A panel in each rhizobox was made of clear acrylic and covered with a black plastic sheet to avoid light exposure. Root images were photographed through the panel at every 20 cm depth. The images were analyzed using the Rootfly root analysis software which is a free, open-source software application to aid researchers in mini-rhizotron image analysis under the GNU General Public License (Stanley and Christina, 2011). The length, diameter and color of roots, as well as numbers alive and dead were recorded. All the experimental data were stored in a single file using the Rootfly software format.

Development of graft union

The graft union formation between 8-month-old rootstocks (clone#5) and scion (RRIM 600) was examined histologically using the standard method with paraffin described below. The samples (measured at 0.5 cm above and below the graft union) were collected at 5, 10 and 20 days after bud grafting (DAB). They were fixed in FAA II (formaldehyde:glacial acetic acid:70% ethyl alcohol; 5:5:90 v/v) for 48 h (Ruzin, 1999). The fixed samples were dehydrated in a tertiary-butyl-alcohol series and embedded in paraffin wax (Histoplast PE; Richard-Allan Scientific, Kalamazoo, MI, USA). Sections (12 μm thick) were cut using a rotary microtome and stained with safranin and fast green (Ruzin, 1999). All sections were observed under a light microscope.

Successful budding percentage

Four weeks after budding, a green bud indicated that budding had been successful. Then, the top of the rootstock was cut to induce shoot emergence. The percentage of successful buds remaining green was determined using Equation (1):

$$\text{Successful budding}(\%) = \frac{\text{Rootstock with successful buds}}{\text{Total number of rootstock}} \times 100 \quad (1)$$

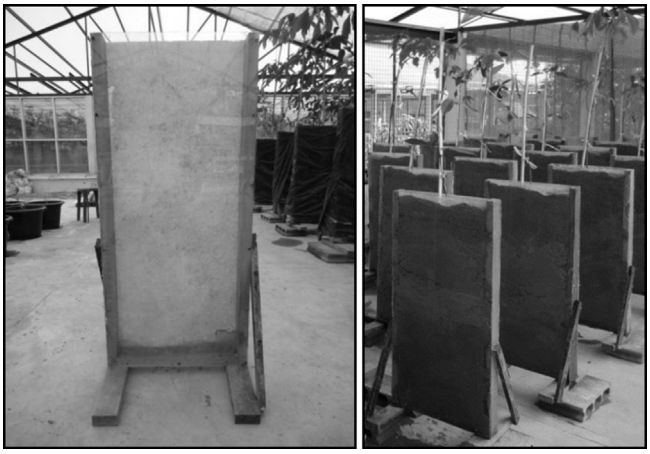

Fig. 1. Rhizoboxes used in the experiment.

Growth of the rubber tree

The plant height was measured at 10 cm from the soil level to the top of the plant shoot. The plant trunk diameter was measured 10 cm from the soil level. The number of leaves was determined by counting the number of compound leaves per plant.

Physiological responses

The photosynthetic rate (A) and stomatal conductance (g_s) were measured for 1000–1200 h using a portable photosynthesis system (LICOR-6400; LI-COR; Lincoln, NE, USA). Measurements were made on three fully expanded leaves for each treatment.

Results and discussion

Root growth of rubber trees

Most of the active root growth in the rhizoboxes was located within 20–40 cm depth from the soil surface with rapid proliferation. Clone#5 and RRIM 600 exhibited high extension root growth to deeper layers of 60–100 cm as shown in Fig. 2. Clone#5 had the highest

Table 1
Location of rubber clones collected in this study.

Name	Coordinates	Place of collection
Clone#1	7° 0′ 23.1″ N 100° 29′ 52.8″E	Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Songkhla
Clone#2	7° 0′ 33.1″ N 100° 29′ 57.2″ E	Khunying Long Athakravisunthon Learning Resources Center, Prince of Songkla University, Hat Yai, Songkhla
Clone#3	7° 0′ 36.0″ N 100° 29′ 53.0″E	Office of the President, Prince of Songkla University, Hat Yai, Songkhla
Clone#4	7° 0′ 37.8″ N 100° 29′ 56.7″ E	Roundabout entrance halls, Prince of Songkla University, Hat Yai, Songkhla
Clone#5	7° 0′ 29.6″ N 100° 30′ 2.2″ E	Sritrang Reservoir, Prince of Songkla University, Hat Yai, Songkhla
RRIM 600	6° 8′ 64.27″ N 100° 42′ 69.38″ E	Klonghoykhong, Rubber Plantation, Songkhla

average root length density followed by clone#2 and these two were significantly different from the other clones as shown in Fig. 3.

Development of graft union

The graft-union was assessed at 5, 10 and 20 DAB by cross cutting at the union of the six cloned rootstocks grafted with the RRIM 600 scion (Fig. 4). The grafting union was assessed at three stages: 1) callus tissues developed on the graft union, with the cambium layers of the scion and stock must be lined up (5 DAB); 2) the graft union growth was almost fully developed with wound areas and callus bridge formation; 3) at 20 DAB to evaluate the percentage of successful budding. Callus formation was observed both on the scion and rootstock, with the new vascular tissue formation being connected with the old cambium and by restoration of the continuity of the epidermis at the graft union.

Successful budding percentage

The percentage of successful budding in rubber varied from 75% to 100%, with 100% success obtained only from clone#4 and RRIM 600 (Table 2).

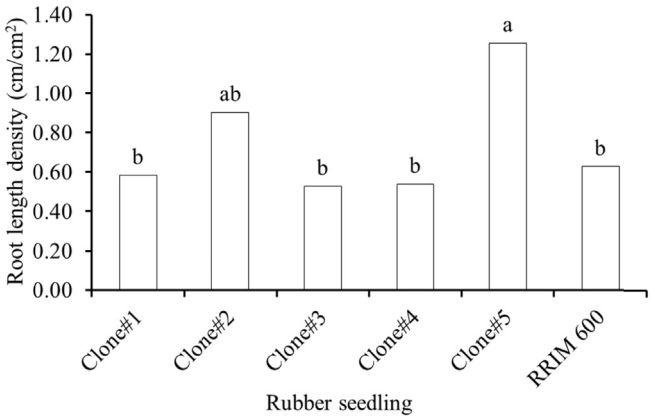

Fig. 3. Average root length densities of the 5 clones compared with the clone RRIM 600 at 3 mth after bud grafting (DAB). Bars with different lower case letters are significantly different at $p \leq 0.05$ by Duncan's multiple range test.

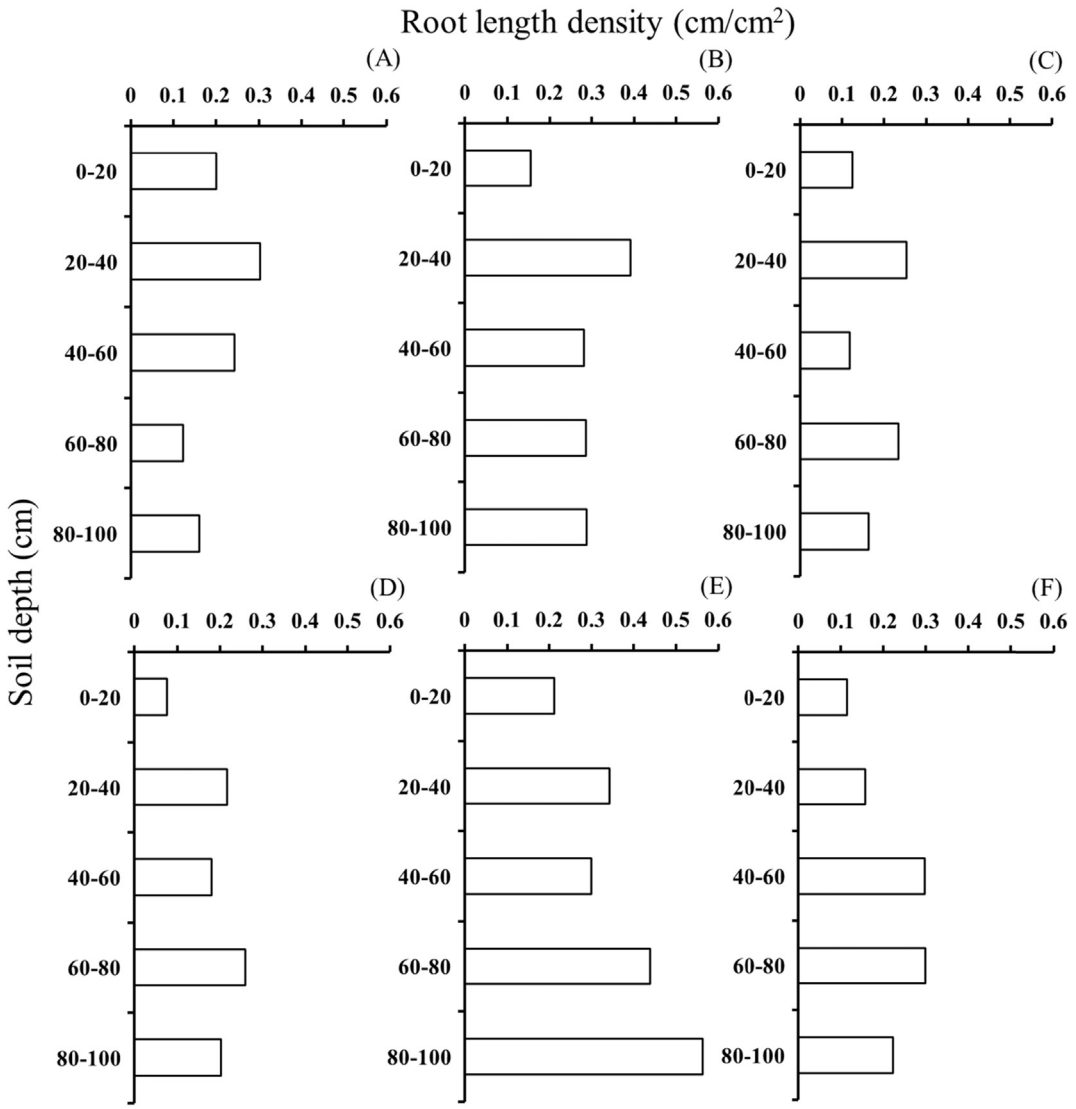

Fig. 2. Root profiles (0–100 cm from soil surface) following budding of five clones compared with clone RRIM 600 at 3 mth after budding: (A) clone#1; (B) clone#2; (C) clone#3; (D) clone#4; (E) clone#5; (F) RRIM 600.

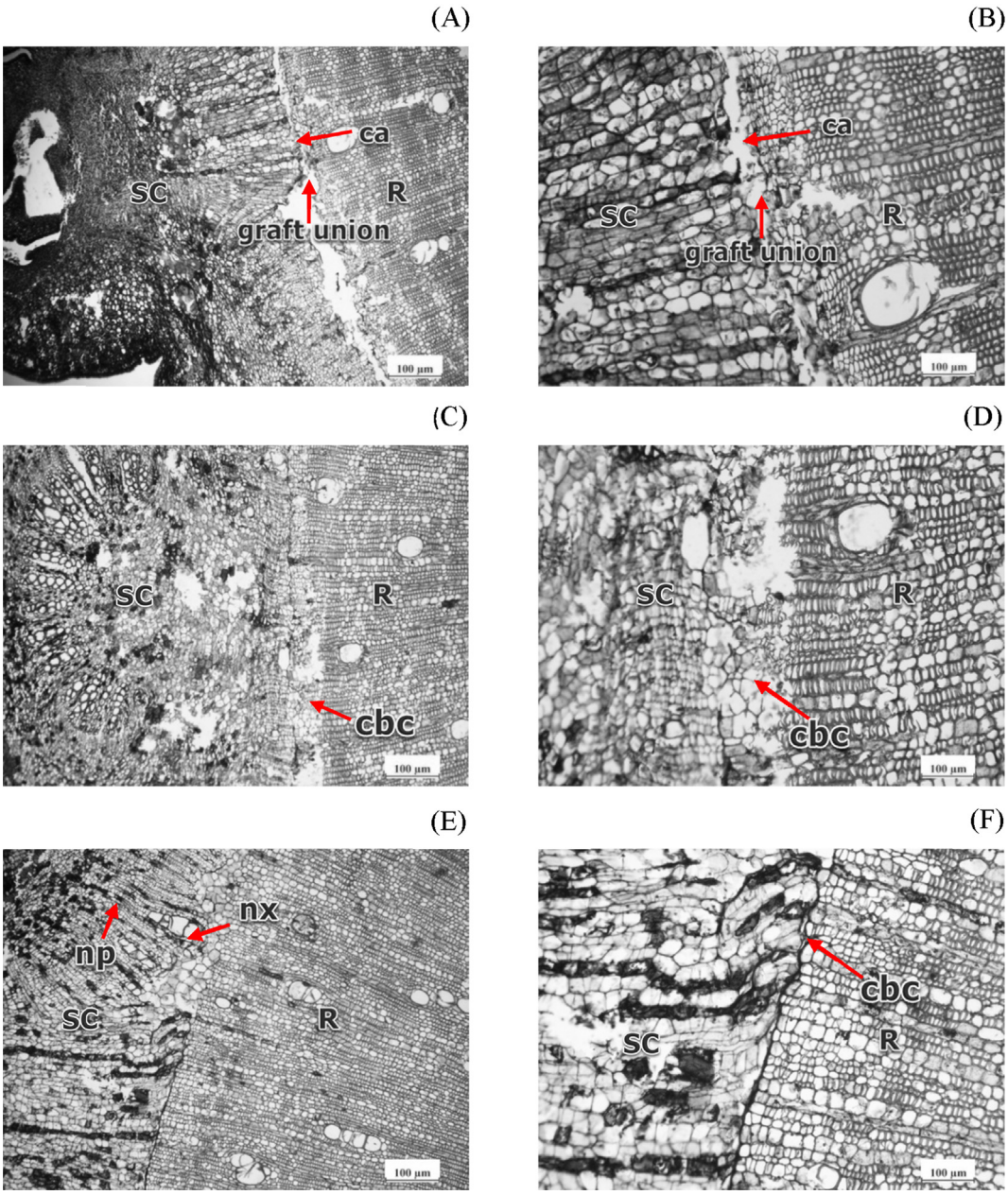

Fig. 4. Graft union development of rootstock clones (clone#5) and RRIM 600 scion: (A) 5 days after budding (DAB); (B) enlargement of (A); (C) 10 DAB; (D) enlargement of (C); (E) 20 DAB; (F) enlargement of (E); SC = scion; R = rootstock; nx = new xylem; np = new phloem; ca = callus; cbc = callus bridge cell.

Early introduced rootstocks with scion growth

The increments of the various clones of rootstocks with scion growth of RRIM 600 after budding for 20 wk were observed. The results showed that the height, diameter and leaf number per plant continuously increased (Fig. 5); clone#5 had the highest height (138.7 cm) and diameter (11.14 mm) but it was not significantly different from the other clones. The RRIM 600 clone had the highest leaf number (35.30 leaves per plant), but it was not significantly different from the other clones.

Table 2
Successful budding percentage in rubber tree clones.

Name	Successful budding (%)
Clone#1	75
Clone#2	75
Clone#3	75
Clone#4	100
Clone#5	75
RRIM 600	100

Physiological responses after budding

Table 3 shows that clone#5 tended to exhibit the highest average photosynthetic rate (7.60 µmol/m^2/s) and clone#4 showed the highest stomatal conductance (243.8 mmol/m^2/s). However, there were no significant differences in the photosynthetic rates and stomatal conductance among the clones (Table 3). The photosynthetic rate and the stomatal conductance of each rubber clone

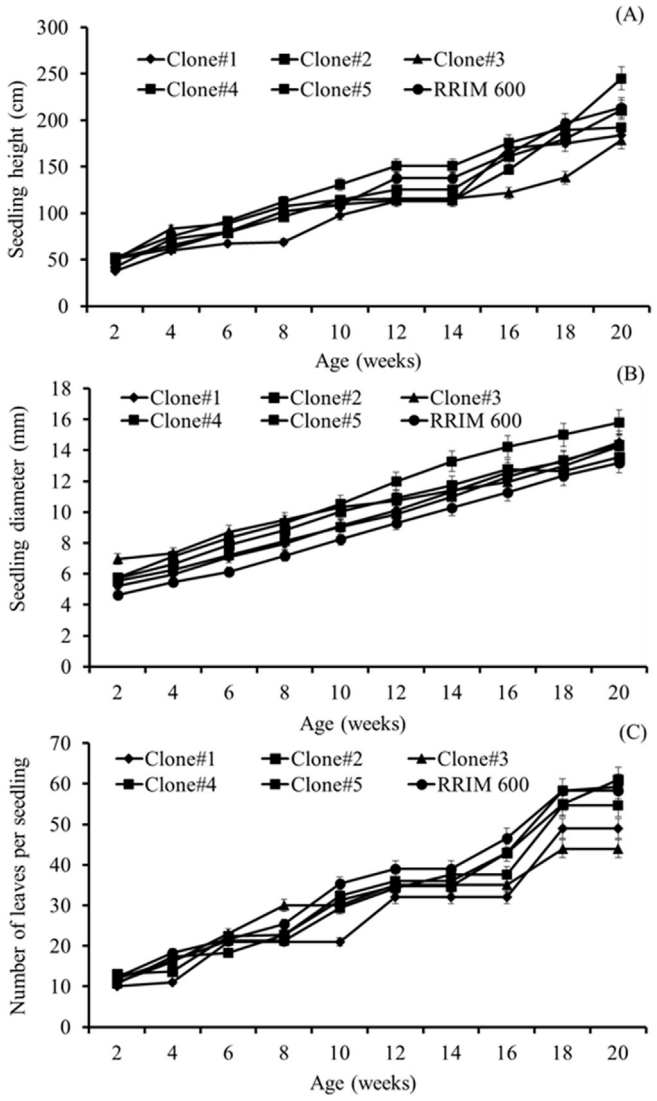

Fig. 5. Scion growth of various rootstocks: (A) height; (B) diameter; (C) leaf number (error bars indicate mean ± SE).

was in the range 6.5–7.5 μmol/m^2/s and 200–250 mmol/m^2/s, respectively.

The soil profile study indicated that most of clones exhibited high root proliferation at 20–40 cm depth. Only clone#5 and RRIM 600 exhibited a high root proliferation in lower layers (60–100 cm depth). Cherngchalard (2012) found that rubber seedlings grown in a mini-rhizotron had high root proliferation at 20–40 cm depth from the soil surface. In an older rubber plantation, George et al. (2009) reported root proliferation in the deeper layers.

Table 3
Photosynthetic rate and stomatal conductance 6 mth after grafting of rubber rootstocks with RRIM 600 scion.

Rootstock of planting material	Photosynthetic rate (μmol/m^2/s)	Stomatal conductance (mmol/m^2/s)
Clone#1	7.58	241.2
Clone#2	7.40	236.4
Clone#3	6.76	219.8
Clone#4	7.19	243.8
Clone#5	7.60	230.2
RRIM 600	7.19	228.0
F-test	ns*	ns
Coefficient of variation (%)	20.26	23.06

*Not significantly different ($p \leq 0.05$) by Duncan's multiple range test.

The success of budding of RRIM 600 on the various clones indicated that there was high compatibility, and there were no significant differences among the clones. Gonçalves and Martins (2002) reported that the scion of RRIM 600 showed high compatibility with various rootstock clones.

Assessment of the grafting union showed that cell division at an early stage involved small, non-uniform cells. Callus development is an important process for the development of the cambial bridge and of new vascular tissue connecting with the old cambium and vascular tissue between the scion and rootstock (Jeffree and Yeoman, 1983). Normally, cell division for the development of parenchyma cells occurs 1–4 DAB (Estrada-Luna et al., 2002). At 10 DAB, callus developed rapidly between the grafting wounds and was completed within 20 DAB. Watson (1989) also reported that the success of budding could be evaluated 18–20 DAB; this was similar to the results of budding success in the current experiment.

The investigation of physiological response supported the success of budding, because the measurement of the stomatal conductance and photosynthetic rate showed a high response. This implied that the sap flow in the vessel tissue promoted transpiration and photosynthesis. Somjun (2009) also reported that a physiological response of rubber could be assessed via the stomatal conductance. It was found that there was a small difference in the stomatal conductance and photosynthetic rate among the clones. Supacharoenkun (2008) reported that the differences in the photosynthetic rate and stomatal conductance also depended on the different clones.

Most of the early introduced rubber clones exhibited active root growth through the soil profile. However, the highest root length density was found in clone#5. Callus tissue developed on the graft union at 5 DAB. The graft union growth was almost fully developed in the wound areas and callus bridge formation was visible at 10 DAB. The new vascular tissue formation was connected with the old cambium and the restoration of continuity of the epidermis at the graft union was evident at 20 DAB. The percentage of successful budding varied from 75% to 100% and the average photosynthetic rate and stomatal conductance were not significantly different among the rubber rootstocks.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission and the Graduate School, Prince of Songkla University, Thailand.

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