

Waterlogging and restricted-below ground aeration on photosynthetic performance and root elongation rate of rubber clones (*Hevea brasiliensis* Mull. Arg.)

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Abstract. Due to extended inundation, waterlogging and restricted below-ground aeration cause inhibition in plant growth performance. This study examined two rubber clones, RRIM 600 and RRIT 251, for waterlogging and restricted below ground aeration. The net photosynthetic rate (PN), maximum quantum yield photosystem II (FV/FM) and root elongation rate were observed to evaluate the plant performance under the stress conditions. RRIM 600 has a higher photosynthetic performance under normal conditions. However, the PN rate and FV/FM ratio trend showed that RRIM 600 seems to have difficulties recovering after exposure to restricted-below ground aeration. Although RRIT 251 had a lower tendency of PN rate under normal conditions, the PN rate and FV/FM ratio of this clone showed fast recovery. RRIT 251 also performed a higher trend of root elongation rate under both stress conditions than RRIM 600.

Keywords: Rubber tree, P_N , F_v/F_m , root elongation rate

INTRODUCTION

Hevea brasiliensis, the para rubber tree provides 99% of the global natural rubber. It covers the south Amazon river area, extending towards the west of Peru and the south to Bolivia and Brazil [1]. The most extensive rubber tree cultivation producing natural latex is located in Southeast Asian countries like Thailand, Malaysia, and Indonesia. The success of rubber cultivation is affected by climatic conditions. Climatic variables can significantly change the cycle of hydrology which cause increases in mean annual precipitation of approximately 200 mm/year after 1971 [2; 3]. Waterlogging caused by high and unpredictable rainfall due to climate change may affect the growth performance of rubber trees.

As abiotic stress, waterlogging negatively affects the productivity of the crop. Hypoxic stress due to the lack of oxygen level in the soil is mainly caused by waterlogging. With the lack

of dissolved oxygen level in the ground, root respiration shifts to the anaerobic mode, consequently reducing root physiological metabolism [4], and water and nutrient absorption by root [5], resulting in crop physiological drought [6] followed by a reduction in leaf photosynthetic rate [7; 8]. When suffering from hypoxic stress, the physiological metabolism of plant roots slows down, and the ability to absorb water is affected [9].

Waterlogging causes restricted below-ground aeration as one factor that inhibits leaf photosynthetic performance due to low soil oxygen levels [10; 11; 12]. Previous work using a standard infrared gas analyzer (IRGA)-based gas exchange analysis indicated that flooding might damage the photosynthetic apparatus [13]. Hypoxia stress due to waterlogging will slow photosynthesis, with implications for lower CO₂ concentrations in the leaf, a potential promoter of stomatal closure [9].

This experiment was conducted to determine how different rubber clones of RRIM 600 and RRIT 251 respond to waterlogging and hypoxia due to restricted below-ground aeration. Currently, RRIM 600 is cultivated in most of the rubber cultivation areas in Thailand, as in

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the past, RRIT 251 was used to be the most dominant clone to be grown by local farmers. This study hypothesized that clones respond differently to inundation and restricted-below ground aeration.

METHODOLOGY

Experimental location and materials

This study was conducted in the nursery house of the Horticulture Department of Kasetsart University, Bangkok-Thailand (latitude: 13.85°N, longitude: 100.75°E) for three months, from August to October. Seven-month-old RRIM 600 and RRIT 251 grafted rubber clones from Nongkhai Rubber Research Station were tested. All rubber seedlings were transplanted into root boxes filled with Pak Chong soil. The ambient air temperature was recorded by using a weather station, WatchDog model 900 ET. During the experiment, the mean ambient temperature was 30°C, relative humidity was 70.45%, and maximum solar radiation was 14.25 KJ m⁻² day⁻¹. The growing condition was designed into three shapes: control (the plants were watered two times a day until the soil reached field capacity), waterlogging and restricted below ground aeration. Restricted below ground aeration was designed by sealing the whole root box area to block the growing chamber's air circulation. The treatments ended when the photosynthetic rate reduced up to 90% from the starting point in each treatment.

Net photosynthetic rate measurement

Portable Photosynthesis System, LI-6400XT (LiCor. Inc), was utilized to measure the net photosynthetic rate ($n = 5$). Leaf photosynthetic rate was calculated on fully developed leaves from 9.00 am to 12.00 pm. CO₂ concentration was set at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, light intensity in the chamber was set at 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The CO₂ flow rate was adjusted to 500 $\mu\text{mol s}^{-1}$, relative humidity in the leaf chamber was 60%, and the temperature chamber was set at 25°C.

Maximum quantum yield photosystem II (F_v/F_m)

F_v/F_m ($n = 5$) was measured using FluorPen (PSI Inc.) The measurement was conducted after 30 minutes of dark adaptation. Saturating light was given at 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, actinic light at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and measuring light at 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Root elongation rate

The grafted rubber seedlings were transplanted to root boxes filled with Pak Chong soil series. Root boxes ($n = 5$) were constructed from iron

frames, supporting vertically orientated transparent plastic glass screens (100 cm length, 40 cm width, 0.5 cm thickness) placed on the surface of the iron frame of the minirhizotron. After one month of transplanting, the rubber tree's roots emerged from the soil to the surface of the root box. Incremental root length extension was recorded every three days by tracing over roots visible at the root boxes screen with different colours of a permanent marker pen and then scanned root tracing. Analyzed root length for each measurement session using image analysis software (WinRHIZO Tron, regent Instruments, Canada).

Experimental design

A randomized complete block design with five replication was applied in this experiment. Two rubber clones were tested as the first factor. Three different growing conditions were used as the second factor: normal condition (control), waterlogging and restricted below ground aeration. Data from each parameter is presented as mean $\pm$ SE. A student t-test was performed using MS excel (Microsoft Windows) to test for different responses between two rubber clones under different conditions.

RESULTS AND DISCUSSION

RRIM 600 is the most planted rubber clone in Thailand as it has high latex productivity [14]. RRIM 600 was also reported to have higher total biomass and C organic levels than GT 1 clone [15]. This study also found that RRIM 600 rubber clone performed higher photosynthetic performance than RRIT 251. However, RRIM 600 showed a different response of net photosynthetic rate, chlorophyll fluorescence and root elongation rate than RRIT 251 when it was exposed to hypoxia stress due to restricted below ground aeration. In this experiment, restricted below ground aeration was set to describe hypoxia conditions that may occur if the waterlogging duration is extended. Soil O₂ level was not measured in this study.

RRIM 600 rubber clone performed a higher trend of photosynthetic rate under normal conditions and particular duration of waterlogging than RRIT 251

To compare the photosynthetic performance of two rubber clones: we measured and analyzed the net photosynthetic rate and chlorophyll fluorescence of RRIM 600 and RRIT 251 under normal conditions, waterlogging and restricted below ground aeration. Our data shows that rubber seedling has a net photosynthetic rate (PN) ranging from 8 to 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Under

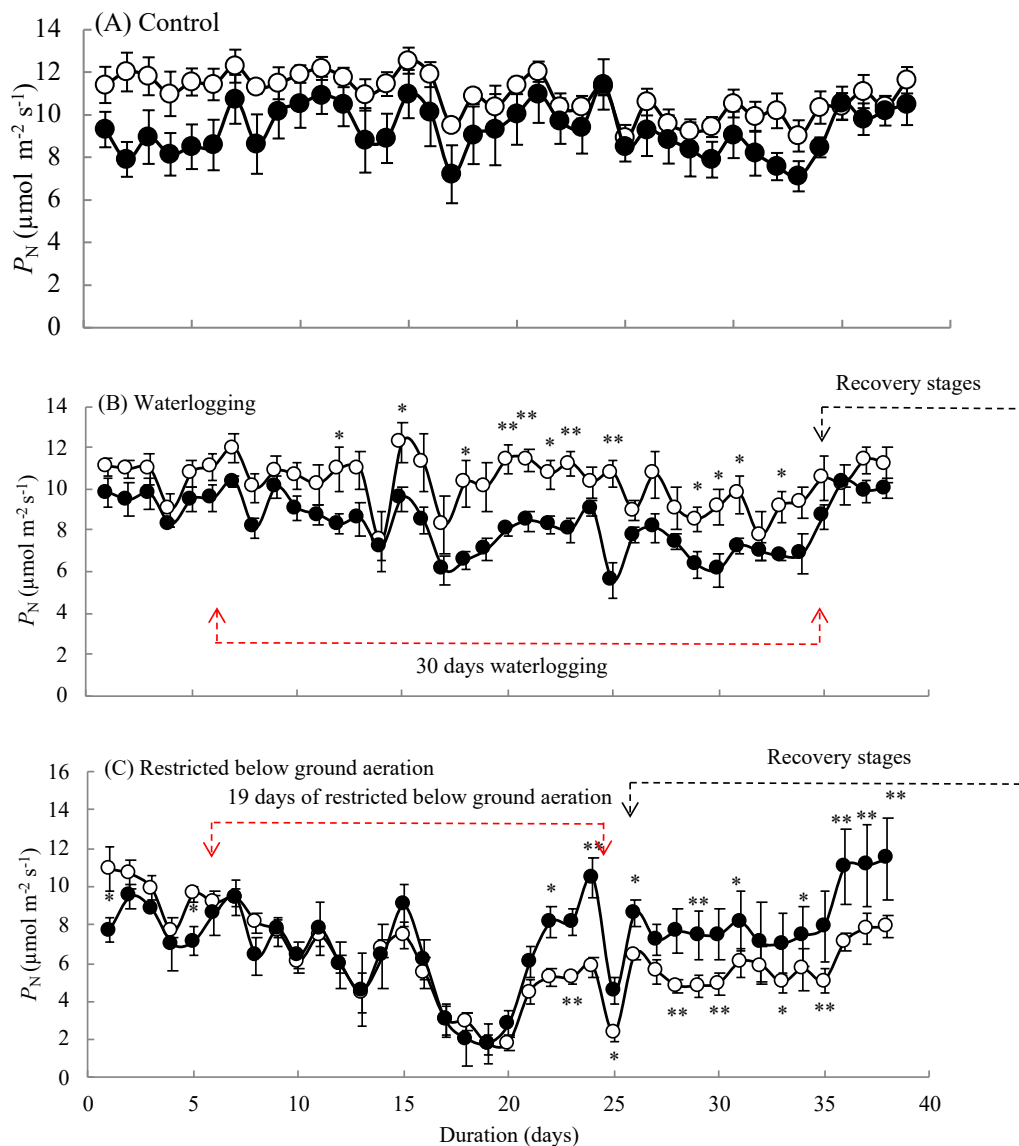


Figure 1. Net photosynthetic rate of RRIM 600 (○) and RRIT 251 rubber clones (●) under (A) normal conditions (control), (B) waterlogging, and (C) restricted below ground aeration. * and ** indicate significant differences at the 0.05 and 0.01 probability levels, respectively, according to the Student t-test. Data corresponds to mean value and standard error (n = 5).

normal conditions, RRIM 600 performed a more dominant P_N trend than RRIT 251 (Fig. 1A). As the plants were exposed to waterlogging for 30 days, the P_N rate of both clones fluctuated, with the lowest P_N rates recorded at 6 and 7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for RRIT 251 and RRIM 600, respectively (Fig. 1B).

Waterlogging and drought conditions cause a similar problem for plants as both conditions will cause water deficiency in plant cells. A former study with RRIM 600 and RRIT 251 rubber clones reported that both clones had similar responses to water shortage conditions. However, stomatal conductance of RRIM 600 rapidly decreased at 42 $\text{mmol m}^{-2} \text{s}^{-1}$ after eight

days of withholding water. While stomatal conductance of RRIT 251 decreased at 10 $\text{mmol m}^{-2} \text{s}^{-1}$ after ten days of withholding water [16]. However, it was not explained how fast both clones could be recovered after the stress.

For the photosynthesis process, CO_2 is absorbed by leaves and then turns into biomass. RRIM 600 performed a higher P_N rate under normal and waterlogged conditions indicating more elevated CO_2 absorbed for photosynthesis. It can be assumed that RRIM 600 is a potential clone to be cultivated to achieve higher growth and yield and also for carbon credit as part of the Clean Development Mechanism (CDM) of

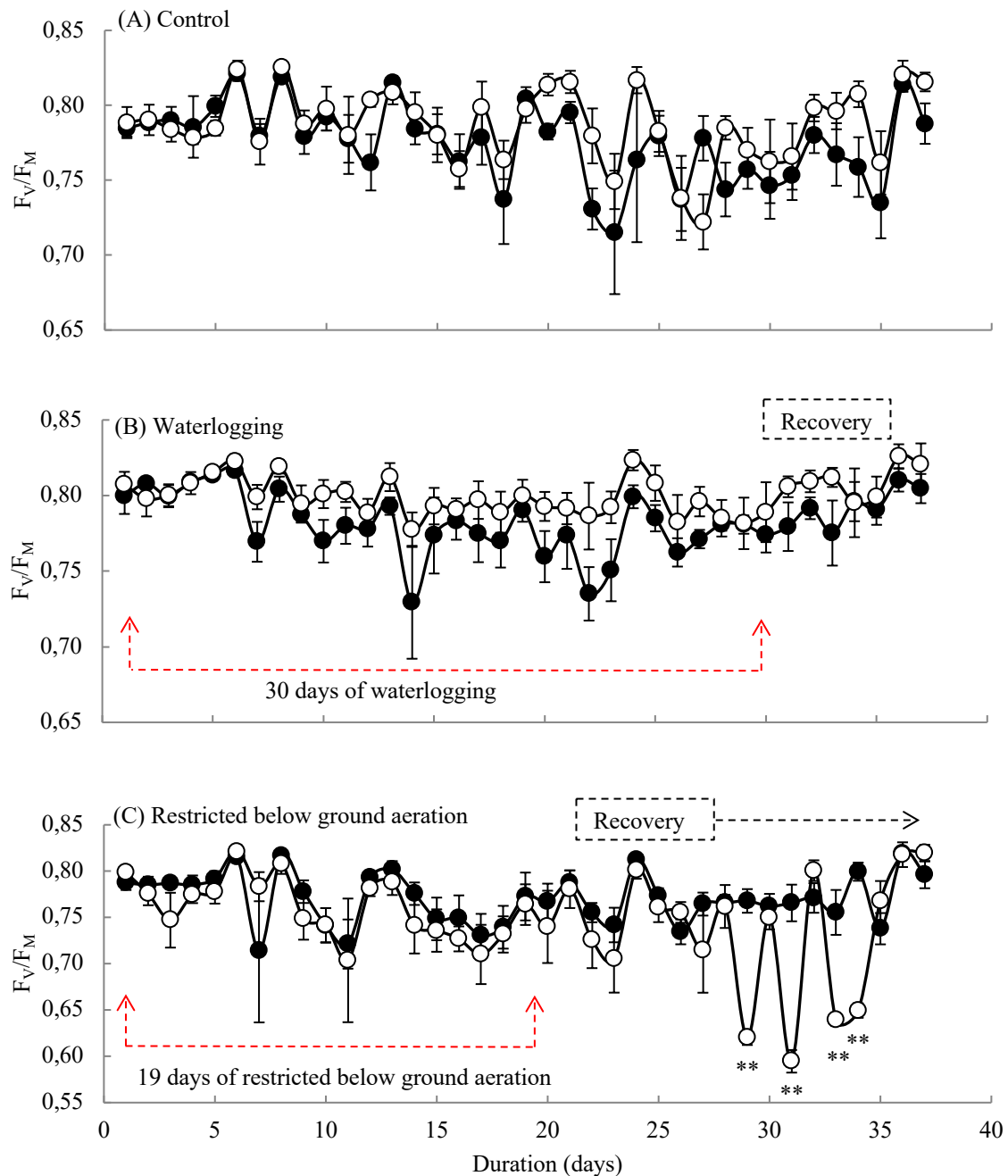


Figure 2. Maximum quantum yield PSII (F_v/F_m) of RRIM 600 (○) and RRIT 251 rubber clones (●) under (A) normal conditions (control), (B) waterlogging, and (C) restricted below ground aeration. ** indicate significant difference at the 0.05 and 0.01 probability levels according to the Student t-test. Data correspond to the mean value and standard error ($n = 5$).

the Kyoto Protocol [17; 18]. Although, in this experiment, we found that RRIM 600 showed a higher P_N rate than RRIT 251, however, it was reported that there was no difference in carbon content between RRIM 600 and RRIT 251 [18].

Photosynthesis of RRIT 251 showed faster recovery after restricted below ground aeration treatment.

Clone RRIT 251 can maintain faster recovery after restricted below ground aeration treatment than RRIM 600, supporting its tolerance of hypoxia conditions. Both rubber clones were exposed to restricted below ground aeration for 19 days, and the results showed that both P_N rates of rubber clones decreased to the lowest level at $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 1C). Before the

treatment, the P_N rate of RRIM 600 was recorded at $11 \mu\text{mol m}^{-2} \text{s}^{-1}$, while during the recovery stage, the P_N rate was recorded at $7.6 \mu\text{mol m}^{-2} \text{s}^{-1}$. It is alleged that the photosynthetic value of this RRIM 600 had difficulty returning to its initial level until the end of the experiment (Fig. 2C). A different response was shown by clone RRIT 251. This clone's photosynthesis could recover faster than RRIM 600 after exposure to 19 days of restricted below ground aeration treatment than RRIM 600. The P_N performance of RRIT 251 was better under this condition than RRIM 600. Before the treatment, the P_N rate of RRIT 251 was recorded at $9.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, while during the recovery stage, the P_N rate was recorded at $10.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 1C).

In normal conditions, the maximum quantum yield of PSII (F_v/F_m) value of both RRIM 600 and RRIT 251 rubber clones ranged from 0.72 to 0.82 (Fig. 2A). Although the F_v/F_m value from both clones was not affected by waterlogging, RRIM 600 showed a higher F_v/F_m value trend than RRIT 251 throughout waterlogging (Fig. 2B). Restricted below ground aeration did not affect F_v/F_m of RRIT 251. The F_v/F_m ratio of RRIT 251 ranged from 0.71 to 0.83 during restricted below ground aeration treatment. F_v/F_m of RRIM 600 was inhibited under restricted below ground aeration. The F_v/F_m value of RRIM 600 decreased to 0.6 after the treatment, indicating a disturbance of light energy absorption in the PSII reaction center. The F_v/F_m ratio of RRIM 600 increased to 0.82 at the end of the experiment (Fig. 2C).

The net photosynthetic rate of RRIM 600 could not recover as fast as RRIT 251 under restricted below ground aeration. The disturbance of P_N during the recovery stage was also stated by a lower F_v/F_m ratio trend under restricted below ground aeration. F_v/F_m ratio is a sensitive indicator to indicate plant photosynthetic performance [19], derived from non-invasive indicators provided by chlorophyll fluorescence which tell us the status of the photosynthetic reaction center in harvesting light energy [20; 21]. Stress plants show an F_v/F_m ratio of around 0.6 after a long period of stress [19], while the F_v/F_m of healthy plants is about 0.74 - 0.85 [22].

This experiment could not detect plant stress under waterlogging using F_v/F_m ratio. It was reported that F_v/F_m could not be used as an excellent indicator to detect pressure in Barley due to waterlogging and drought [23]. A similar result was reported that photosynthetic performance on hybrid peach trees under water restriction and waterlogging was also undetectable using F_v/F_m . The value remained

constant over the day of drought and waterlogging [24].

RRIT 251 performed a higher root elongation rate under waterlogging and after restricted below ground aeration treatment.

The mechanism of plants to respond to waterlogging is complex. Currently, morphological and physiological adaptations that occur due to hypoxia stress are well understood. Morphological modifications cover the formation of lateral roots [25], development of aerenchyma tissue [26]. In contrast, physiological changes include decreasing net photosynthetic rate during hypoxic stress [27]. Morphological modification results a tolerant plants that can develop structures that allow oxygen diffusion to occur up to the root tips [28].

In normal conditions, the trend of root elongation rate of RRIM 600 was higher than RRIT 251. There was an increase in root elongation rate for both clones from the beginning to the end of the experiment, although the increasing trend was not constant. There were some periods when the root elongation rate increased and decreased. The root elongation rate reduction occurred several days before the root elongation rate increased (Fig. 3A).

RRIM 600 and RRIT 251 clones showed a rapid increase in root elongation rate under 30 days of waterlogging. When waterlogging ended, the root elongation rate of both clones tended to slow. One of the plant mechanisms to access the soil area with higher O_2 levels is by increasing the root growth performance. The root elongation rate of RRIT 251 remained higher than RRIM 600 in the recovery stage of waterlogging and until the end of the experiment (Fig. 3B). Similar to waterlogged conditions, under restricted below ground aeration, the root elongation rates of RRIT 251 were higher than RRIM 600 over the treatment. A higher trend of root elongation rate allows plants to reach areas with higher O_2 levels in the soil, contributing to faster recovery of leaf photosynthetic performance.

Under the stress conditions, the rubber clones tend to have a higher root elongation rate than normal conditions. It may help the plants access other areas where O_2 is still available. Different depths of waterlogging also affect the spatial distribution of root growth [29]. In the recovery stages, visually, we observed and found that the plant produced new roots rather than growing the existing sources. A similar response was reported in chickpea; release from waterlogging enhanced new root growth rate than regrowth of

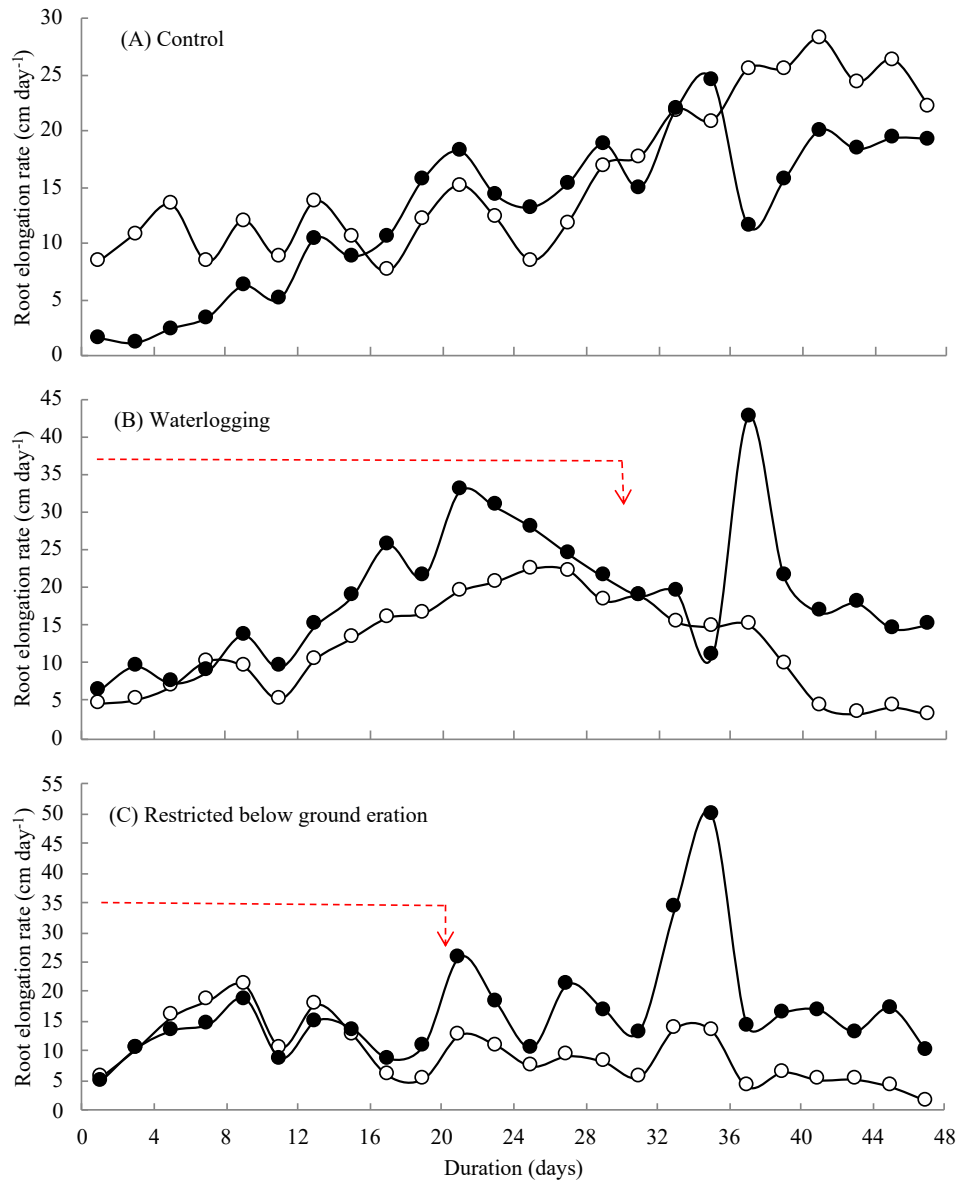


Figure 3. Root elongation rate of rubber genotype of RRIM 600 (○) and RRIT 251 (●) under normal conditions (A), waterlogging (B), and restricted below ground aeration (C). Red lines indicate stress duration. Data corresponds to mean value ($n = 5$).

existing roots [30]. The ability of the plant to maintain glycolysis and fermentation in root respiration under hypoxic stress may help explain its tolerance to waterlogging [28].

CONCLUSION

Although the photosynthetic performance and root elongation rate of RRIM 600 are more dominant than RRIT 251, RRIM 600 is considered more susceptible to hypoxia stress. It was indicated by the slow recovery process of the clone indicated by lower net photosynthetic rate, chlorophyll fluorescence, and root elongation in the recovery stages. The net photosynthetic performance of RRIT 251 is

lower than RRIM 600 in normal conditions. However, this clone could recover immediately from hypoxic stress, indicated by the plant's ability to recover the net photosynthetic rate and chlorophyll fluorescence to the initial value before the stress treatment started.

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