The Stomatal Conductance and Fv/Fm as the Indicators of Stress Tolerance of Avocado Seedlings under Short-Term Waterlogging

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Abstract: Avocados may suffer from short-term waterlogging stress when exposed to high temperatures and heavy rainfall during the summer in Taiwan. We compared the waterlogging responses of own-rooted and grafted seedlings of two Taiwan cultivars, ‘Black-Beauty’ and ‘Hung-Hsin-Yuan’, by stomatal conductance (gs) and chlorophyll fluorescence parameters. Four-day waterlogging and four-day post-waterlogging recovery periods were investigated. Both gs and Fv/Fm of own-rooted seedlings of two cultivars were significant reductions in response to short-term waterlogging. The grafted seedlings on the same cultivar rootstock were evaluated by gs and Fv/Fm during the growth and the growth cessation periods, respectively. The combined responses of gs and Fv/Fm under short-term waterlogging showed that ‘Black-Beauty’ was sensitive to stress because of decreased gs after waterlogging or decreased Fv/Fm after the two-day recovery period. ‘Hung-Hsin-Yuan’ showed more tolerance to waterlogging stress, especially during the growth cessation. This indicates that the vegetative dormancy may affect the evaluation of the stress response of avocados. Our results revealed that gs and Fv/Fm can be effective indicators in the four-day waterlogging of avocado, and the growth status of avocado seedlings should be considered during stress-tolerant variety selection.

Keywords: Persea americana; flooding; own-rooted; grafting

1. Introduction

Avocado (Persea americana) is grown worldwide for its oil-rich fruit. It has recently become an important crop in Taiwan, and the area under cultivation has increased rapidly in the past decade. The cultivation areas of avocado are mainly in southern Taiwan. Taiwan is located in the tropical and subtropical monsoon climate zone. In summer, there are typhoons or temporary heavy rains, causing short-term waterlogging problems in orchards. Many avocado cultivars in Taiwan are bred locally for desirable fruit characteristics and stable production [1]. However, the avocado nursery system in Taiwan generally uses the own-rooted avocado seedlings as rootstocks rather than using rootstock cultivars with specific traits. The seedling rootstocks are typically grown from the processed seeds of avocado fruits.

Waterlogging is a complex environmental pressure that is perceived as several distinct stress types, including hypoxia, changes in soil pH, and increased pathogen activity, all of which contribute to the overall stress experienced by plants [2]. This complex stress results in reduced photosynthesis, stomatal closure, and diminished root water conductivity, leading to reductions in growth and yield [3]. A reduction in stomatal conductance (gs) is often an early response, which is probably attributable to a decline in root hydraulic
conductance and is often more pronounced in waterlogging-susceptible plants [4]. The multifactorial nature of waterlogging stress thereby poses considerable challenges from the perspective of developing commercially important waterlogging-tolerant crop varieties [5].

Avocado trees are shallow-rooted, the roots of which generally extend to depths of within 60 cm below the surface and are sensitive to waterlogging, with soil hypoxia due to short-term waterlogging initiating stress responses [2,6]. The responses of avocado to waterlogging often manifest as plant withering, leaf fall, growth inhibition, and root browning and necrosis [2,7]. Waterlogging stress causes reductions in chlorophyll content and CO₂ assimilation rate, and leads to changes in stomatal conductance and photosynthesis-related Fv/Fm values [8,9]. Moreover, soil hypoxia affects leaf net gas exchange and chlorophyll fluorescence parameters [10]. The performance of different cultivars in response to waterlogging could be gauged by assessing the performance of the above-ground parts, which would enable the rapid screening for variability among cultivars [11,12].

Given recent trends of an increasing incidence of short-duration heavy rainfall, as a consequence of extreme weather events [13], climate change has increased not only the likelihood of sudden intense rainfall but also the potential exposure of plant-differing combinations of environmental stress [14]. In this regard, whereas in temperate species that are dormant during winter, trees can often overcome waterlogging stress via adaptive bud protection mechanisms [15,16]. Avocado trees, as subtropical fruit trees, only have growth cessation but not dormancy during winter. However, the different performance in response to the growing season and winter when avocado trees suffer from the waterlogging has, to date, been insufficiently studied.

Many findings have pointed out that the waterlogging tolerance of fruit trees may be dependent on the rootstock [17,18]. However, when comparing the growth vigor, biomass, and the leaf fluorescence from the scion of different cultivars that were grafted on the rootstock of the same cultivar, the physiological characteristics of the scion were affected by the stress responses during the waterlogging tolerance [4,12]. Taiwan is located in a typhoon-affected area, with an average of five typhoons hitting Taiwan every year. In addition to the wind damage caused by the typhoon, another serious impact is a large amount of rainfall in a short period, which causes short-term waterlogging in the orchard. Therefore, the prevention of short-term waterlogging is an important issue for the Taiwan avocado industry.

In Taiwan, avocado production is characterized by the utilization of a given cultivar as either a scion or a rootstock. Therefore, we selected two local cultivars that are commonly cultivated in Taiwan, which are ‘Black-Beauty’ and ‘Hung-Hsin-Yuan’, to investigate the respective performances of own-rooted and grafted seedlings of the same cultivars under short-term waterlogging and during post-waterlogging recovery by measuring gs and chlorophyll fluorescence parameters. We compared the short-term stress response during the growing status and the growth cessation to find out whether the growth status affects the stress response. The aim of this research was to explore the waterlogging tolerance between local cultivars and to provide a reference for the evaluation of avocado seedling development in Taiwan.

2. Materials and Methods

2.1. Plant Materials and Waterlogging Evaluations

One-year-old own-rooted seedlings of ‘Black-Beauty’ (BB) and ‘Hung-Hsin-Yuan’ (HHY), as well as one-year-old grafted seedlings of the two cultivars with own-rooted ‘Chang-An’ seedlings as the rootstock, were obtained from the same nursery. The plants were planted in peatmoss as the main medium. Given that in the avocado-producing areas of Taiwan, short-term waterlogging generally occurs in summer, the waterlogging evaluations were conducted in the phytotron with day/night temperatures of 35/30 °C and natural light.
The preliminary test was conducted for two to seven days regarding the waterlogged condition during the typhoon attacks. The relevant differences were shown by different avocado varieties after four days of waterlogging. Therefore, we chose four days of waterlogging as the experimental duration. The seedlings were divided into waterlogging and normal irrigation (control) groups. The waterlogging treatment was to completely submerge the seedlings with water above the soil surface, and the water level was about 5 cm higher than the pot. Irrigation of the seedlings in the control group was conducted at 2-day intervals until the soil was fully hydrated, as confirmed by observations of the dampness of the soil surface. Own-rooted seedlings were evaluated to confirm the stress response parameters. Grafted seedlings were assessed in the different growth statuses: once each was during the growing season in spring and the growth cessation was in winter, respectively.

The investigation period was four consecutive days with treatments (W0 to W4), followed by a recovery period in that the waterlogged seedlings were removed from waterlogging stress and continuous observation for a further four consecutive days (R0 to R4). For each cultivar, each treatment was performed with three replicates. During both the waterlogging and post-waterlogging periods, all parameters were measured at 2-day intervals.

2.2. Determinations of Stomatal Conductance and Chlorophyll Fluorescence Parameters

Photosynthesis parameters were measured using the fourth leaves from the terminal bud on each plant. Stomatal conductance (gs) was measured at three points on each leaf using an SC-1 stomatal conductance meter (METER Group, Inc., Pullman, WA, USA) between the hours of 10:00 and 12:00.

Chlorophyll (Chl) fluorescence parameters were taken from a healthy fully expanded leaf on three plants by three repetitive per treatment between 22:00 and 00:00 by using a Junior-pam chlorophyll fluorometer (Heinz Walz GmbH Co., Effeltrich, Germany). Specifically, Fo and Fm were measured in a light-protected environment in order to facilitate the calculation of Fv/Fm, photochemical fluorescence quenching coefficient (qP), nonphotochemical quenching coefficient (qN), and nonphotochemical quenching (NPQ). The measuring leaves were the same leaves that were used for gas-exchange determinations.

2.3. Statistical Analysis

Test data were subjected to analysis of variance (ANOVA) using CoStat 6.451 statistical software (Cohort Software, Monterey, CA, USA). When ANOVA revealed significant differences, pairwise comparisons were performed using Fisher’s least significant difference (LSD) test. Line plots were generated using Sigmaplot 14.0 statistical software (Systat Software, San Jose, CA, USA).

3. Results

3.1. Stomatal Conductance

We observed reductions in the gs of both BB and HHY own-rooted seedlings in response to an initial exposure to waterlogging (Figure 1). After 4 days of short-term waterlogging (W4), the gs of seedlings in the waterlogging group significantly decreased, with reductions of up to 57.7% and 56.7% for BB (Table 1) and HHY (Table 2), respectively. When measured 4 days after the recovery stage from waterlogging (R4), the gs of BB and HHY own-rooted seedlings remained at the low level with 59% and 48% of the pre-waterlogging, respectively, and was still significantly lower than that of the control group seedlings (Tables 1 and 2). Similarly to the own-rooted seedlings, the gs of both BB and HHY-grafted seedlings had a reduction following exposure to waterlogging (Figure 2). At the W4 stage, there was a significant difference in gs between the waterlogging and control groups, with reductions of up to 76.7% and 84.7% being recorded for BB and HHY-
grafted seedlings, respectively (Tables 1 and 2). Moreover, until the R4 stage, the gs of grafted seedlings was found to have remained at low levels (Figure 1A,F).

Figure 1. The stomatal conductance (gs) and chlorophyll fluorescence parameters of two cultivar own-rooted seedlings during waterlogging (W0, W2, and W4) and the post-waterlogging recovery period (R0, R2, and R4). (A–E) Performance of cultivar ‘Black-Beauty’ (BB). (F–J) Performance of cultivar ‘Hung-Hsin-Yuan’ (HHY). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments on the same investigated day.
Table 1. Stomatal conductance (gs), Fv/Fm, and Fo of avocado cultivar ‘Black-Beauty’ during 4-day waterlogging and 4-day post-waterlogging periods.

<table>
<thead>
<tr>
<th>Seedlings</th>
<th>Treatments</th>
<th>W0</th>
<th>W2</th>
<th>W4(R0)</th>
<th>R2</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Own-rooted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Waterlogged</td>
<td>142.21 a</td>
<td>152.93 a</td>
<td>57.82 b</td>
<td>106.49 b</td>
<td>132.95 b</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>142.13 a</td>
<td>149.04 a</td>
<td>136.84 a</td>
<td>219.12 a</td>
<td>225.66 a</td>
</tr>
<tr>
<td></td>
<td>Grafted</td>
<td>178.14 a</td>
<td>126.09 a</td>
<td>31.24 b</td>
<td>24.82 c</td>
<td>26.44 c</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>168.84 a</td>
<td>136.14 a</td>
<td>133.80 a</td>
<td>191.82 a</td>
<td>277.77 a</td>
</tr>
<tr>
<td></td>
<td>Fv/Fm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Own-rooted</td>
<td>0.738 a</td>
<td>0.672 c</td>
<td>0.662 c</td>
<td>0.668 b</td>
<td>0.668 bc</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.741 a</td>
<td>0.713 bc</td>
<td>0.732 b</td>
<td>0.710 a</td>
<td>0.731 a</td>
</tr>
<tr>
<td></td>
<td>Grafted</td>
<td>0.767 a</td>
<td>0.738 ab</td>
<td>0.765 a</td>
<td>0.633 c</td>
<td>0.618 c</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.758 a</td>
<td>0.768 a</td>
<td>0.781 a</td>
<td>0.706 a</td>
<td>0.730 ab</td>
</tr>
<tr>
<td></td>
<td>Fo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Own-rooted</td>
<td>551.3 a</td>
<td>452.0 a</td>
<td>587.0 a</td>
<td>595.0 a</td>
<td>473.0 b</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>540.7 a</td>
<td>429.0 ab</td>
<td>418.0 b</td>
<td>562.0 a</td>
<td>417.3 b</td>
</tr>
<tr>
<td></td>
<td>Grafted</td>
<td>339.3 a</td>
<td>320.0 b</td>
<td>339.3 c</td>
<td>468.3 b</td>
<td>606.7 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>333.3 a</td>
<td>327.3 b</td>
<td>329.0 c</td>
<td>408.0 c</td>
<td>400.7 b</td>
</tr>
</tbody>
</table>

*: W0, W2, and W4 represent 0 days, 2 days, and 4 days after waterlogging, respectively. R0, R2, and R4 represent the post-waterlogging treatment for 0 days, 2 days, and 4 days, respectively. †: Different lowercase letters indicate significant differences among the treatments of the own-rooted seedlings and grafted seedlings by LSD (p < 0.05).

Table 2. Stomatal conductance (gs), Fv/Fm, and Fo of avocado cultivar ‘Hung-Hsin-Yuan’ during 4-day waterlogging and 4-day post-waterlogging periods.

<table>
<thead>
<tr>
<th>Seedlings</th>
<th>Treatments</th>
<th>W0</th>
<th>W2</th>
<th>W4(R0)</th>
<th>R2</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Own-rooted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Waterlogged</td>
<td>113.05 a</td>
<td>81.32 a</td>
<td>44.48 c</td>
<td>56.75 bc</td>
<td>56.70 bc</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>113.99 a</td>
<td>114.36 a</td>
<td>102.66 b</td>
<td>87.96 b</td>
<td>116.92 b</td>
</tr>
<tr>
<td></td>
<td>Grafted</td>
<td>167.63 a</td>
<td>94.93 a</td>
<td>26.19 c</td>
<td>23.03 c</td>
<td>25.69 c</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>176.37 a</td>
<td>145.55 a</td>
<td>171.03 a</td>
<td>236.97 a</td>
<td>195.66 a</td>
</tr>
<tr>
<td></td>
<td>Fv/Fm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Own-rooted</td>
<td>0.743 a</td>
<td>0.699 b</td>
<td>0.707 b</td>
<td>0.671 b</td>
<td>0.674 b</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.758 a</td>
<td>0.746 ab</td>
<td>0.760 a</td>
<td>0.748 a</td>
<td>0.755 a</td>
</tr>
<tr>
<td></td>
<td>Grafted</td>
<td>0.779 a</td>
<td>0.772 a</td>
<td>0.773 a</td>
<td>0.762 a</td>
<td>0.736 ab</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.768 a</td>
<td>0.722 ab</td>
<td>0.776 a</td>
<td>0.686 ab</td>
<td>0.709 ab</td>
</tr>
<tr>
<td></td>
<td>Fo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Own-rooted</td>
<td>460.0 a</td>
<td>454.0 a</td>
<td>496.3 a</td>
<td>466.3 a</td>
<td>390.3 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>465.0 a</td>
<td>376.7 b</td>
<td>389.0 b</td>
<td>427.0 ab</td>
<td>373.7 a</td>
</tr>
<tr>
<td></td>
<td>Grafted</td>
<td>270.0 c</td>
<td>290.7 c</td>
<td>306.7 c</td>
<td>346.0 c</td>
<td>384.0 a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>337.7 b</td>
<td>357.0 b</td>
<td>323.3 bc</td>
<td>378.7 bc</td>
<td>380.0 a</td>
</tr>
</tbody>
</table>

*: W0, W2, and W4 represent the 0 days, 2 days, and 4 days after waterlogging, respectively. R0, R2, and R4 represent the post-waterlogging treatment for 0 day, 2 days, and 4 days, respectively. †: Different lowercase letters indicate significant differences among the treatments of the own-rooted seedlings and grafted seedlings by LSD (p < 0.05).
Figure 2. The stomatal conductance (gs) of grafted seedlings of ‘Black-Beauty’ (BB) (A,B) and ‘Hung-Hsin-Yuan’ (HHY) (C,D) treated with waterlogging (W0, W2, and W4) during the growth period or the growth cessation and continuously investigating the post-waterlogging recovery period (R0, R2, and R4). Bars indicate the standard deviation of three replicates, and the different letters represent the significant difference between the treatments on the same investigated day.

3.2. Chlorophyll Fluorescence Parameters

At the end of the waterlogging treatment (W4), the chlorophyll fluorescence parameter Fv/Fm of own-rooted avocado seedlings detected reductions of approximately 7% to 10% (Tables 1 and 2). The value did not return to the initial levels, even after 4 days of post-waterlogging recovery (Figure 1B,G). In the case of the grafted seedlings, the two assessed cultivars were found to show differential responses to waterlogging. Notably, the Fv/Fm of grafted BB seedlings detected no reductions during the period of waterlogging, although the values started to decline during the post-waterlogging period, down to 81% at the R4 stage (Table 1). In contrast, the Fv/Fm had no significant differences in grafted HHY seedlings during and after the removal of waterlogging (Figure 3). Therefore, it indicated that grafted HHY seedlings can effectively maintain their chlorophyll fluorescence reactions when exposed to waterlogging stress.
Figure 3. The Fv/Fm of grafted seedlings of ‘Black-Beauty’ (BB) (A,B) and ‘Hung-Hsin-Yuan’ (HHY) (C,D) treated with waterlogging (W0, W2, and W4) during the growth period or the growth cessation and continuously investigating the post-waterlogging recovery period (R0, R2, and R4). Bars indicate the standard deviation of three replicates, and the different letters represent the significant difference between the treatments on the same investigated day.

After 4 days of waterlogging, Fo values increased for the own-rooted seedlings of both cultivars, which differed significantly from Fo in the control group (Tables 1 and 2). After the removal of waterlogging stress, Fo was found to have declined to the same levels as the control group levels (Tables 1 and 2). Contrastingly, there were no significant differences between waterlogging and control group own-rooted seedlings of either cultivar with respect to other photosynthesis-related chlorophyll fluorescence parameters, including qP (Figure 1C,H), qN (Figure 1D,I), and NPQ (Figure 1E,J), under waterlogging or during the post-waterlogging period. The results of the own-rooted seedlings indicated that Fv/Fm is a better index to evaluate the short-term waterlogging effect of avocado seedlings among all Chl fluorescence parameters.

3.3. The Waterlogging Response of Grafted Seedlings in Different Growth Statuses

The phenology of avocado in Taiwan flushes dominantly in spring and summer, and ceases vegetative growth in winter. The seedling reproduction is mainly in winter. To examine the growth difference of gs and Fv/Fm in grafted avocado seedlings under short-term waterlogged conditions, we evaluated grafted BB and HHY seedlings during the growth period and the growth cessation period, respectively.

Our observations indicated that compared with the control group seedlings, gs in grafted BB seedlings during the growth period started to show significant reductions at the W4 stage, with gs levels remaining low throughout the post-waterlogging period (Figure 2A). Comparatively, when measured during the growth cessation period, although gs in grafted BB seedlings also declined in response to waterlogging, the decline was consid-
erably more rapid (Figure 2B). The differences relative to the control group became significant after 2 days of waterlogging (R2), and again, gs remained at low levels, which was only 7% of the control group value, throughout the post-waterlogging period, failing to recover to normal levels at the end of the evaluation period. Grafted HHY seedlings during the growing period showed similar trends of gs variation with BB seedlings. The values had significant declines and failed to recover, ultimately reaching levels of only 13% of the control group at the end of the post-waterlogging period (Figure 2C). In contrast, the gs of grafted HHY seedlings evaluated during the growth cessation period did not differ significantly from those of control group seedlings (Figure 2D). It indicated that waterlogging stress may not pose a threat to grafted HHY seedlings when the growth stopped.

We also detected differences between the two cultivars with respect to Fv/Fm. The Fv/Fm of grafted BB seedlings showed a sustained decline during the growth period as long as the waterlogging started, but the significant decline was shown at the W4 (R0) stage during the growth cessation (Figure 3A,B). The post-waterlogging reduction in Fv/Fm during the growth cessation was found to be 39% relative to that of the control group, which was higher than the 15% reduction during the growth period. Contrastingly, the grafted HHY seedlings, during both the growth or growth cessation periods, showed no significant differences in Fv/Fm relative to the control group during either the waterlogging or post-waterlogging periods (Figure 3C,D). However, the slight variation during the whole evaluation period indicated that the effects of waterlogging on Fv/Fm of grafted HHY seedlings were weak in response to waterlogging stress, especially during the growth cessation period.

4. Discussion

The stomatal conductance has a direct influence on plant water relationships and photosynthesis [14]. Lower vapor pressure deficits (VPDs) caused by the high temperature of enclosed environments may decrease the rates of leaf evapotranspiration [19], and it may prevent a rapid recovery of gs, even after an alleviation of waterlogging stress. Accordingly, the experimental day/night temperatures of 35/30 °C in the phytotron could be the reason that the reduced levels of gs failed to recover after the removal of waterlogging stress. Moreover, the light source of the phytotron is natural light. The stomatal conductance would increase in response to an increase in light intensity [20,21]. Under transient changes in light intensity, plants tend to spend a considerable amount of time adjusting stomatal apertures to attain a new state of equilibrium [22].

The dark-adapted Fv/Fm and Fo vary slightly during normal growth, while Fv/Fm undergoes a continuous decline and Fo increases during the light inhibition [4,23]. It makes these two chlorophyll fluorescence parameters serve as reliable indicators of stress [24,25]. Having been relieved of waterlogging stress, plants may experience a rapid transition from a state of hypoxia to one of normal oxygen supply, with the influx of oxygen leading to an accumulation of reactive oxygen species, thereby resulting in oxidative stress [26,27]. The simultaneous exposure to strong light during this phase may cause photo-oxidative damage to plant photosystems, which will further reduce the rate of photosynthesis [28]. As the gs and chlorophyll fluorescence parameters are often used as indicators of stress [29,30] and the stress response of own-rooted seedlings of the two cultivars we evaluated under the short-term waterlogging, the two parameters, the gs and Fv/Fm, are effective indexes for evaluating the avocado short-term waterlogging tolerance of Taiwanese cultivars.

It has previously been demonstrated that, whereas Fv/Fm in the root rot-resistant rootstocks ‘Dusa’ and ‘Duke7’ show differential reductions when these two varieties are exposed to waterlogging stress, there are no differences with respect to qP and NPQ [31]. In the present study, we found that when subjected to short-term waterlogging, whereas grafted BB and HHY seedlings both showed similar percentage reductions in gs, grafted BB seedlings showed a higher percentage reduction in Fv/Fm (Tables 1 and 2; Figures 2
and 3). The results indicated that cultivar ‘Black-beauty’ may have a poorer capacity to recover from short-term waterlogging at high temperature after grafting, and that even short-term waterlogging may have a detrimental effect on its photosynthetic efficiency. The maintenance of energy production is a key facet in the response of avocado to waterlogging [32], and a reduction in photosynthetic capacity would predictably render avocados more susceptible to the threat of waterlogging [12].

When comparing the stress responses between grafted seedlings under the growth and growth-stopped period, two cultivars showed different recovery potentials to the short-term waterlogging. In gs results, BB and HHY both showed a decrease after 4-day waterlogging during growth, but only BB’s gs declined at W2 during growth cessation; HHY had no different response between the treatment and control group when seedlings were during growing cessation (Figure 2). BB showed a significant difference in Fv/Fm at the recovery period (R2) for seedlings both during growth or growth cessation; however, there was no difference in the Fv/Fm of HHY-grafted seedlings between the treatment and control group during any growth situation (Figure 3). Combining the results of gs and Fv/Fm, BB is more sensitive to short-term waterlogging, and the stress response occurs earlier. HHY has the potential to survive if it encounters short-term waterlogging during the period of growth cessation. In the research of the conifer responding the flooding, it shows different responses of Fv/Fm when waterlogging occurs at the growing season or at the dormant season, and the higher tolerance was found in dormant conifers [33]. Our finding showed that although subtropical trees such as avocado do not have a dormant phase of growth that is characterized in temperate woody plants, the seedling tends to show more protection when facing the flooding stress during the growth cessation. Therefore, the growth status of avocado should be taken into consideration when assessing waterlogging tolerance using stress response indicators.

Our results show that it is indeed possible to distinguish the waterlogging tolerances of different varieties within 4 days of short-term waterlogging. This level of waterlogging not only allows breeders to shorten the entire breeding schedule and have a rapid screening strategy, but it is also in line with the waterlogged state of avocado mostly encountered in summer in Taiwan.

5. Conclusions

Avocados may suffer from short-term waterlogging stress when exposed to high temperatures and heavy rainfall during the summer in Taiwan. The own-rooted seedling is used to be the rootstock and the grafted seedling is the producing plants. Understanding the difference of the stress response of two local commercial cultivars is beneficial to the avocado industry in Taiwan. In this study, the effective photosynthetic indicators to short-term waterlogging under high temperature of own-rooted seedlings of both cultivars, ‘Black-Beauty’ and ‘Hung-Hsin-Yuan’, were gs and Fv/Fm. These two indicators were also effective at showing the different waterlogged tolerance for the grafted seedlings of the two cultivars during the growth cessation. It also revealed the effect of the growth status on the evaluation of waterlogging tolerance in avocado.

Consequently, the stomatal conductance and Fv/Fm can be effective indicators to evaluate the short-term waterlogging tolerance of the avocado seedlings. The avocado cultivar, which is sensitive to waterlogging, reveals a significant decline response within four-day waterlogging. Our findings could help establish an effective and short-term screening strategy for flooding-tolerant varieties of avocados in subtropical regions.

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**References**


