

# Comparison of Chlorophyll Fluorescence of Three Citrus Rootstocks and Satsuma Mandarin Grafted on Them

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**ABSTRACT** Leaf chlorophyll fluorescence capable to estimate CO<sub>2</sub> assimilation was compared among three citrus rootstocks: trifoliolate orange (*Poncirus trifoliolate* L.), 'Flying Dragon' (*Poncirus trifoliolate* L. var. *monstrosa*) and citromelo (*Poncirus trifoliolate* L.×*Citrus paradisi*), as well as among satsuma mandarin (*Citrus unshiu* cv. Nichinan No.1) grafted on the 3 rootstocks. Citromelo, the most vigorous rootstock; and 'trifoliolate orange'; the most common cultivar and moderate rootstocks gave the same potential and actual quantum yields, whereas 'Flying Dragon' (very dwarf) had lower values due to a higher Fo and Fs for fluorescence obtained from dark-adapted and light-adapted leaves, respectively. These findings show that the absorbed photon energy was dissipated more as fluorescence from the antenna chlorophyll in 'Flying Dragon' than trifoliolate orange or 'swingle' citrimelo. The satsuma mandarins grafted on these rootstocks did not, however, show the differences observed in the rootstocks by having all the same potential and actual quantum yields. It is suggested that the rootstocks do not potentially or actually electron transport in the Photosystem II (PS II) of satsuma mandarin grafted on the 3 rootstocks.

**Additional key words:** quantum yield, electron transport, photosystem II

## Abbreviations

Fo, minimum fluorescence yield in the dark-adapted state; Fs, fluorescence yield in the steady state during illumination; Fm, maximum fluorescence yield during illumination; Fv=Fm-Fo, variable fluorescence; Ft, fluorescence at any giventime;  $\Phi_p^{open}=(Fm-Fo)/Fm$ , potential quantum yield;  $\Phi_p=(Fm'-Fs')/Fm'$ , actual quantum yield.

## Introduction

Citriculture in Korea and Japan has depended on a single rootstock cultivar, 'trifoliolate orange', because satsuma mandarin, the most common citrus cultivar in both countries, when grafted on this rootstock grows to a moderately vigorous and a very disease resistant and bears high quality fruits. Use of a variety of rootstocks, however, should help to solve present problems and prevent future ones. Much of works about the relationships between rootstocks and scions were performed from morphological approach (Castle et al., 1980; Constantin et al., 1979; Hutchison, 1977; Phillips, 1977; Takahara et al., 1994; Wheaton et al., 1991; Wutscher and Shull, 1976, Wutscher and Dube, 1977). It is important to evaluate performance of CO<sub>2</sub> assimilation and carbohydrate partitioning of the satsuma mandarin grafted. Nevertheless, there is few reports concerning carbohydrate content and performance of photosynthetic characteristics of 'trifoliolate orange' because it is very difficult to measure photosynthesis of the trees due to their

small seized leaves. However, the fact that chlorophyll fluorescence measurement can give the PS II activity is indispensable for driving the Calvin-Benson cycle (Foyer et al., 1990). It is reported that PSII activity, defined by actual quantum yield ( $\Phi_p$ ), lineally linked to CO<sub>2</sub> fixation in C<sub>4</sub> plants (Khamis et al., 1990; Krall and Edwards, 1992) and correlated with the fixation in C<sub>3</sub> plants. In this experiment, I have investigated whether chlorophyll fluorescence parameters including the actual quantum yield were different in the satsuma mandarin grafted on the 3 rootstocks. On the basis of my findings, I have discussed the state of PS II of different rootstocks and whether the rootstocks can affect the PS II satsuma mandarin scions.

## Materials and Methods

### Plant materials

One-year-old and non-grafted 3 rootstock cultivars, i.e. 'trifoliolate orange' (*Poncirus trifoliolate* L.), 'Flying Dragon' (*Poncirus trifoliolate* L. var. *monstrosa*) and 'Swingle' citrimelo (*Poncirus*

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*trifoliata* L. × *Citrus paradisi*) and 2-year old satsuma mandarin (*Citrus unshiu* cv. Nichinan No.1), grafted on the 3 rootstocks were used. Six trees for each of the rootstock cultivar, and three trees for each of the satsuma mandarin grafted on the 3 rootstocks were used. All were grown in pots and moved to a phytotron maintained at 25°C and 60% relative humidity during the experiment period. Healthy leaves from the spring shoot without secondary shoot growth were sampled and assayed in late June.

#### Chlorophyll fluorescence measurements

A PAM 2000 portable fluorometer (Waltz, Germany) with attachments was used for all the measurements comparing the characteristics of chlorophyll fluorescence between the 3 rootstock cultivars with and without satsuma mandarin scions on the rootstocks. Measurements were made for three leaves rootstock tree and five leaves satsuma mandarin tree grafted on the 3 rootstock cultivars.

For the minimum fluorescence yield ( $F_0$ ) and the maximum fluorescence yield ( $F_m$ ), determinations were made after dark adaptation of the samples.  $F_0$  was sampled on excitation with 650nm of measuring light below  $5 \mu\text{mol m}^{-2} \cdot \text{sec}^{-1}$  at 600Hz modulation from a pulse LED. The  $F_m$  was recorded after emission induced by a saturating pulse (0.1sec) of white actinic light, repeated eight times, to close the reaction center. The variable fluorescence ( $F_v$ ) was calculated by subtracting  $F_0$  from  $F_m$  (Calatayud et al., 1996).  $F_v/F_m$ , which represents maximal photochemical energy dissipation, was regarded as the potential quantum yield ( $\Phi_p^{\text{open}}$ ). After 2 minutes of dark re-adaptation, actinic white light (about  $30 \text{ mol m}^{-2} \cdot \text{sec}^{-1}$ ) was switched on, and saturation pulses were applied at intervals of 20 sec. After every saturation pulse, the maximum fluorescence yield during illumination ( $F_m'$ ) was recorded. The actual quantum yield ( $\Phi_p$ ) was estimated from  $(F_m' - F_s)/F_m'$  (Gentry et al., 1989) when  $F_t$ , fluorescence at any given time, reached the stable state (defined as  $F_s$ ).

Measurement of tree height, shoot length and leaf area

Tree height and average shoot length and average leaf area were measured and calculated. The length of all shoots were measured and the average length was calculated. Twenty leaves from each rootstock and ten leaves from each of the satsuma mandarin grafts on 3 rootstock cultivars were collected and the average leaf areas for each rootstock and satsuma mandarin were calculated. Data shown in Table 1 are analysis of means obtained by statistical analysis with Fisher's PLSD test.

## Result and Discussion

Comparison of tree height, average shoot length and average leaf area

Among three rootstock cultivars, the tree height was smaller for 'Flying Dragon' than 'Swingle' citrumelo or trifoliata orange. The average shoot length and average leaf areas differed significantly in the order of 'Flying Dragon' < trifoliata orange < 'Swingle' citrumelo. The 'Swingle' citrumelo was 3.3 times longer in shoot length and 4.4 times larger in leaf area than 'Flying Dragon' (Table 1).

Among the satsuma mandarin trees grafted on the 3 rootstock cultivars, the tree height of Satsuma mandarin grafted on 'Flying Dragon' or trifoliata orange was smaller than 'Swingle' citrumelo. However, the average shoot lengths and average leaf areas of satsuma mandarin on 3 rootstock cultivars were not different, ranging from 18.9–20.9 cm and 20.2–23.4 cm<sup>2</sup>, respectively (Table 1).

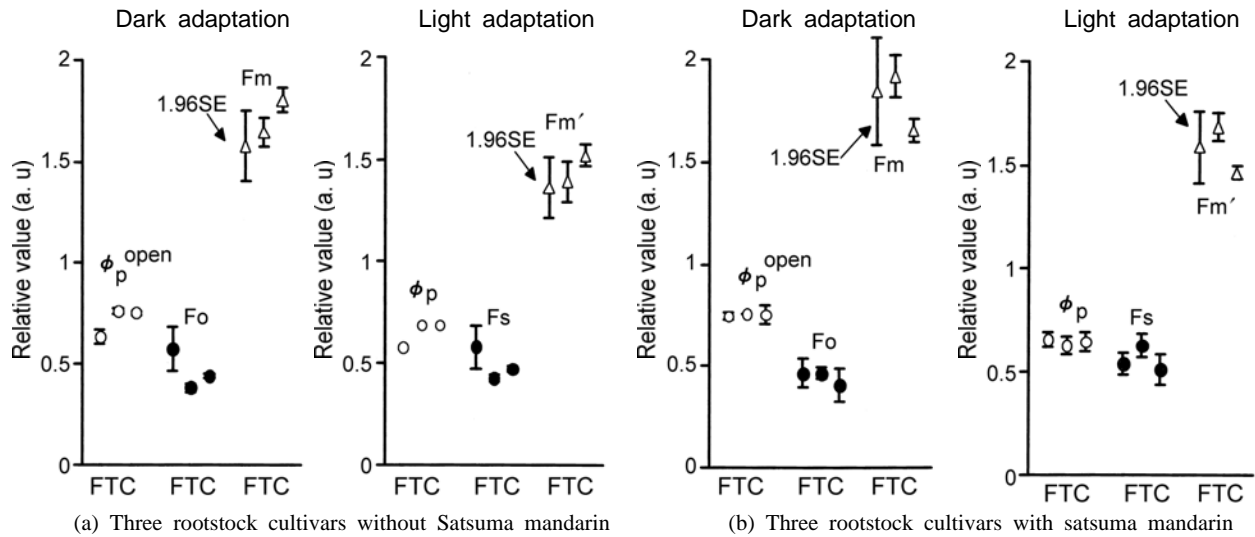
Chlorophyll fluorescence parameters for the three rootstock cultivars

The potential quantum yields ( $\Phi_p^{\text{open}}$ ), represented by  $F_v/F_m$ , were the same for 'Swingle' citrumelo and trifoliata orange by 0.75 and 0.77, respectively, but were significantly higher than the 0.64 for 'Flying Dragon'. 'Flying Dragon' had the higher minimum fluorescence yield in the dark adapted state ( $F_0$ ) value (0.58) than 'Swingle' citrumelo (0.44) or trifoliata orange

**Table 1.** Comparison of tree height, shoot and leaf growth among 3 rootstock cultivars without or with satsuma mandarin scion cultivar grafting.

Treatment	Tree height (cm)	Average shoot length (cm)	Average leaf area (cm <sup>2</sup> )
Rootstocks without satsuma mandarin grafting			
Flying dragon	51.7b <sup>z</sup>	7.9c	4.1c
Trifoliata orange	71.3a	14.9b	5.0b
Swingle citrumelo	66.5a	25.5a	17.9a
Rootstocks with Satsuma mandarin grafting			
Flying dragon	21.0b	18.9a	23.4a
Trifoliata orange	29.5b	20.9a	22.5a
Swingle citrumelo	40.3a	19.0a	20.2a

<sup>z</sup>Within each treatment combination, means, i.e. a mean of 6 trees for each rootstock cultivar and a mean of 3 trees for each Satsuma mandarin tree on different rootstocks, followed by different letters are significantly different by Fishers LSD test,  $P = 0.05$ .



**Fig. 1** Differences in chlorophyll fluorescence parameters of three citrus rootstocks (T: trifoliolate orange, F: 'Flying Dragon' and S: 'Swingle' citrumelo) and satsuma scion cultivar. Vertical bars denote 1.96×standard error. Fo: minimum fluorescence yield in the dark-adapted state; Fs: fluorescence yield in the steady state during illumination; Fm: maximum fluorescence yield; Fm': maximum fluorescence yield during illumination;  $\phi_p^{open}$ : potential quantum yield,  $(F_m - F_o)/F_m$ ;  $\phi_p$ : actual quantum yield,  $(F_m' - F_s)/F_m$ .

(0.38). The maximum fluorescence yield in dark adapted state (Fm) was higher in 'Swingle' citrumelo (1.8) than trifoliolate orange or 'Flying Dragon' (Fig. 1-a).

Differences in each parameter were similar to those found for dark-adapted leaves when Ft became stable after 16 saturation pulses. The actual quantum yields ( $\phi_p$ ), representing the efficiency of electron transport in the PSII, were almost the same for 'Swingle' citrumelo and trifoliolate orange (ca. 0.69) but, they were, significantly higher than the 0.58 for 'Flying Dragon'. Fs was higher for 'Flying Dragon' (0.58) than 'Swingle' citrumelo (0.47) or trifoliolate orange (0.43). Fm' was between 1.4 and 1.5 for all three rootstocks and the order for  $\phi_p$ , Fs and Fm' was the same as that for  $\phi_p^{open}$  and  $\phi_p$  mainly due to significantly higher Fo and Fs. The  $\phi_p^{open}$  and  $\phi_p$ , respectively, represent a potential and actual efficiency of the absorbed photon energy transfer into PS II and the efficiency directly affect on the activity of Calvin-Benson cycle and thereby influenced on CO<sub>2</sub> assimilation (Krall and Edwards, 1992). Also the tree height, the average shoot length and the average leaf area were smallest in 'Flying Dragon'. These imply that the efficiency of electron transport in the PSII is involved on the growth of leaf and/or shoot although the rank of  $\phi_p^{open}$  and  $\phi_p$  were not always consistent with those of average shoot length and average leaf area (Fig. 1-a).

Chlorophyll fluorescence of satsuma mandarin scion cultivar on the three rootstocks

The potential quantum yield ( $\phi_p^{open}$ ) and Fo, respectively, ranged from 0.75–0.76 and 0.41–0.47 for the satsuma mandarin grafted on the three rootstocks did not show differences

among the rootstocks. As for Fm, trifoliolate orange had a higher value (1.92) than 'Swingle' citrumelo (1.65), but did not differ from 'Flying Dragon' (1.85). The parameters observed from the light-adapted leaves showed similar tendency as compared with the parameters from the dark-adapted leaves. The actual quantum yields ( $\phi_p$ ) and Fs of the satsuma mandarin ranged from 0.63–0.66 and 0.52–0.63, respectively, showing no difference among the 3 rootstocks. Fm' in trifoliolate orange (1.68) was higher than 'Swingle' citrumelo (1.46), but did not differ from 'Flying Dragon' (1.59). Unlike the rootstocks, without grafting, there were no differences in  $\phi_p^{open}$  and  $\phi_p$  as there were no differences in average shoot length and average leaf area among scions (Fig. 1-b, Table 1).

The results suggest that the potential ( $\phi_p^{open}$ ) and actual ( $\phi_p$ ) quantum yield correlated with average shoot and leaf growth of the rootstocks do not affect the leaf and shoot growth of the scions. Therefore, the rootstocks do not seem to potentially and actually affect the electron transport in PS II of satsuma mandarin threes grafted on the three rootstocks.

## 감귤 대목과 그것에 접목한 온주밀감의 엽록소 형광특성의 비교

한상헌

일본 과수시험장 감귤부

초 록

3종의 감귤대목 탕자, 비룡, 시트르 메로와 그것에 접목한 온주

밀감 ‘일남 1호’의 CO<sub>2</sub> 동화를 평가할 수 있는 엽록소 형광을 비교하였다. 강세 대목인 시트르 메로와 중간 대목인 탕자는 잠재적이며 실제적으로 거의 같은 광양자 흡수율을 나타냈지만, 약세 대목인 비룡은 암조건과 명조건에서 얻어진 Fo, Fs가 높기 때문에 상대적으로 낮은 광양자 흡수율을 보였다. 이러한 결과는 비룡은 흡수한 광 에너지 안테나 엽록소로부터 형광으로 손실이 되는 율이 높다는 것을 시사하고 있다. 그러나 이들 대목에 접목을 한 온주 밀감은 잠재적이며 실제적으로 거의 같은 광양자 흡수율을 나타냈다. 이러한 사실로부터 대목은 접수의 양자 흡수율에 직접 영향을 미치지 않는다고 사료된다.

추가 주요어 : 광양자 흡수율, 전자전달, 광체제 II

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