Determination of Chilling Sensitivity of Mango (*Mangifera indica* L.) Leaves using Chlorophyll Fluorescence

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Summary

The relationship between low temperature stress and the responses of different mango cultivars 'Haden', 'Irwin', 'Mahachanok' and 'Jin Huang' was monitored on changes in its fluorescence yields as expressed in Fv/Fm through the application of a non-destructive and sensitive technique - chlorophyll fluorescence. Excised mango leaves from leaf positions at 1.1 (3 month-old), 1.2 (6 month-old) and 1.3 (8 month-old) were subjected to seven temperature regimes starting from 25^{o}C, serving as the optimum temperature followed by chilling temperatures at 12^{o}, 9^{o}, 6^{o}, 3^{o}, 1^{o}C and one freezing temperature at -3^{o}C. There was significant decrease in Fv/Fm upon exposure to chilling temperature at 12^{o}C after 24 hours of incubation in the dark. For chilling sensitive cultivar, Fv/Fm values continue to decline with a further drop in temperature but in less-sensitive, chilling tolerant cultivars, their Fv/Fm are statistically tied to values early in the chilling treatment even if the low temperature stress is increased in time and space. Fv/Fm values that were higher than 0.6 were classified as chilling tolerant and values starting from 0.5 and below as chilling sensitive. Hence, 'Haden', 'Irwin' and 'Jin Huang' are chilling tolerant mango cultivars and 'Mahachanok' as chilling sensitive.

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Introduction

The common mango (*Mangifera indica* L.) belongs to the family Anacardiaceae. It is a native plant specie to Asia and is where its center of origin and diversity lies. It is distributed throughout the tropical and several subtropical countries across the continent where it plays an integral part of their economies albeit forming a conspicuous feature in the landscape of the countryside.

Temperatures slightly above freezing (1-12^{o}C) have an adverse effect in the membrane permeability and metabolic activities on many plant species particularly those of tropical origin (Christiansen, 1979). The physical transition of the membrane bilayer to solid-gel state is the turning event in chilling injury physiology followed by impaired metabolic processes, ion movements and protoplasmic streaming after prolonged/continued exposure to chilling temperatures.

The plasma membrane being the first line of defense of plant cells against biotic and abiotic stresses creates a scenario for a start of a cascade of events towards chilling injury. Murata and Los (1997) said that a putative sensor (i.e. His-Kinase protein or Ca^{2+} channel) in the microdomains of the plasma membrane detects a dramatic conformational change upon a downward shift in temperature. Sharom *et al.* (1994) further describes that the exposure to low temperature immobilizes high-melting membrane phospholipids causing them to form separate gel phase domains.

In this study, chlorophyll was used as a method for assessing the level of stress injury that is rapid, sensitive and non-destructive to the tissue (Smillie and Hetherington, 1983). Maxwell and Johnson (2000) further reiterated that the real strength of chlorophyll fluorescence lies in its ability to give insights into the plants capacity to tolerate environmental stresses and the extent to which these stresses have damaged the photosynthetic apparatus even before visible symptoms are evident. Fv/Fm is the frequently used parameter in stress-related studies which foretells the maximum quantum efficiency of the PS II photochemistry (Baker and Rosenqvist, 2004).

Finally, the thermal habitat in which the plants are geographically grown (i.e. origin), the extent of low temperature stress (i.e. time), as well as the plant material – plant or leaf age, leaf detachment or reduced water potential contribute to a decline in Fv/Fm values.

The purpose of this study was to determine the chilling behavior of mango cultivars –'Haden', 'Irwin', 'Mahachanok' and 'Jin Huang' therough the characterization of their Fv/Fm values.

Materials and Methods

I. Plant material

Four mango cultivars 'Haden', 'Irwin', 'Mahachanok' and 'Jin Huang' were used in this study. Mango leaves were collected from 9-10 in the morning at the National Chung Hsing University - grape research center station at Wufong, Taichung county, Taiwan and were immediately transported back to the laboratory for further analysis. The leaves were harvested according to flushing positioning the branch. The youngest, topmost, sun-exposed leaves was the 1st flushing cycle (1.1, 3 month-old), and 1.2 (6 month-old) and 1.3 (oldest, 8 month-old) corresponds to the 2nd and 3rd flushing cycles, respectively.

II. Chilling treatment

Detached leaves were cleaned using moistened tissue paper to remove surface dirt on the mango leaves. The middle portion of the leaf was excised and 1 cm guide circles were made on parallel sides in between the midvein so that successive measurements shall be collected on the same part of each leaf. In order to minimize water loss, the plastic petriplates were lined with two layers of tissue paper and added with 3 ml pure water. Leaf samples were subjected to seven temperature treatments: 25°, 12°, 9°, 6°, 3°, 1°, and -3°C. Stored in a low temperature incubator in the dark with daily measurements beginning at 0 hours and subsequently at 24, 48, 72, 96, and 120 hours. Four leaf replicates were used for each treatment.

III. Chlorophyll fluorescence

Fv/Fm was measured using a Mini-PAM fluorometer (Walz, Effeltrich, Germany). With each daily measurement of the chlorophyll fluorescence, the samples were temporarily removed from the dark environment in the refrigerator. They were immediately measured for fluorescence yield (Fv/Fm) under white fluorescent light at ambient room temperature (25°C). Eight petriplates at-a-time were removed for measurement which corresponds to two cultivars per temperature treatment.

Mean values from four replicates for each treatment were compared by DMRT at the 5% level of significance. Statistics software package SAS 9.1 (Institute, Inc., USA) was used to process the data.

Results

Subsequent chilling at 12°C showed a dramatic decrease in Fv/Fm was observed for all cultivars but was lowest for 'Mahachanok' (Table 1.). A progressive decline in Fv/Fm values as the temperature treatment decreased but was significantly lowest at freezing at -3°C for 'Irwin' and 'Mahachanok', respectively. For 'Jin Huang', the Fv/Fm showed recovery starting from 6°C (0.681) and from 3° to -3°C (0.724) was fully restored to values as in 25°C or sometimes higher.

Table 1. Fv/Fm values between temperature and cultivar.

Temperature (°C)	Fv/Fm			
	'Haden'	'Irwin'	'Mahachanok'	'Jin Huang'
25	0.748 a*	0.747 a	0.747 a	0.720 ab
12	0.662 c	0.675 bc	0.584 de	0.675 bc
9	0.662 c	0.668 c	0.598 d	0.670 c
6	0.666 c	0.682 b	0.611 c	0.681 bc
3	0.662 c	0.653 c	0.579 de	0.724 a
1	0.659 c	0.658 c	0.575 de	0.706 ab
-3	0.702 ab	0.545 de	0.327 e	0.724 a

* Means with the same letter are not significantly different by DMRT. P=0.05.

** Means collectively derived from all flushing position. 1.1 (youngest, 3 month-old), 1.2 (6 month-old) and 1.3 (oldest, 8 month-old).

*** After 120 hrs of storage

At 25°C the chlorophyll fluorescence between flushing positions showed that the older leaves at 1.2 and 1.3 (Table 2.) had statistically higher Fv/Fm values than younger leaves collected from position 1.1 (youngest). Although subsequent exposure to chilling temperature starting from 12°C until 1°C showed significant decrease in Fv/Fm, the values remained statistically tied between

chilling treatments. Only at -3°C did the Fv/Fm registered at 0.568 (position 1.2), 0.554 (position 1.3) and 0.600 (position 1.1) compared to values at chilling temperatures (i.e. 12°, 9°, 6°, 3°, 1°C) that were above 0.6.

Table 2. Fv/Fm values between temperature and flushing position.

Temperature (°C)	Fv/Fm		
	1.1**	1.2	1.3
25	0.716 ab*	0.756 a	0.749 a
12	0.646 c	0.648 c	0.653 c
9	0.645 c	0.627 cd	0.676 bc
6	0.653 c	0.668 c	0.660 c
3	0.650 c	0.643 c	0.649 c
1	0.664 c	0.653 c	0.631 c
-3	0.600 d	0.568 d	0.554 d

* Means with the same letter are not significantly different by DMRT. P=0.05.

** Flushing position on the branch. 1.1 (youngest, 3 month-old), 1.2 (6 month-old) and 1.3 (oldest, 8 month-old).

The chlorophyll fluorescence between cultivar and flushing position (Table 3.) showed that 'Jin Huang' had higher Fv/Fm at flushing positions 1.2 and 1.3 which corresponds to older leaf ages. While 'Mahachanok' in the same positions showed the lowest. For 'Irwin', Fv/Fm were higher at positions 1.1 and 1.2. For 'Haden', Fv/Fm showed significant differences between flushing positions but was lowest at 1.2 and highest at 1.1.

The effect of storage period on the different mango cultivars (Table 4.) showed that after 24 hours of low temperature storage at chilling temperature starting from 12°C, there was a significant decrease in Fv/Fm in all four cultivars. 'Jin Huang' had statistically maintained higher Fv/Fm values

until 120 hours compared to 'Haden', 'Irwin' and 'Mahachanok'. Meanwhile, 'Mahachanok' had the lowest values starting from 48 hours with a decreasing trend until 120 hours.

After 24 hours of storage at chilling temperature from 12^{o}C, Fv/Fm showed a significant decrease. Another significant decline in fluorescence yield was observed after 72 hours at 12^{o}C, 96 hours and 120 hours at 9^{o}C (Table 5.).

Table 3. Fv/Fm values between cultivar and flushing position.

Flushing Position	Fv/Fm			
	'Haden'	'Irwin'	'Mahachanok'	'Jin Huang'
1.1	0.692 ab*	0.656 cd	0.599 de	0.667 b
1.2	0.660 bc	0.655 cd	0.566 e	0.724 a
1.3	0.674 b	0.673 b	0.557 e	0.709 a

* Means with the same letter are not significantly different by DMRT. P=0.05.

** Means collected after 120 hrs of final storage at -3^{o}C.

Table 4. Fv/Fm values between cultivar and storage period.

Storage Period (hr)	Fv/Fm			
	'Haden'	'Irwin'	'Mahachanok'	'Jin Huang'
0	0.774 a*	0.765 a	0.772 a	0.779 a
24	0.631 cd	0.644 bc	0.631 cd	0.665 bc
48	0.681 bc	0.652 bc	0.576 cd	0.691 b
72	0.663 bc	0.635 cd	0.516 d	0.689 bc
96	0.634 cd	0.638 c	0.485 d	0.685 bc
120	0.667 bc	0.631 cd	0.465 d	0.690 bc

* Means with the same letter are not significantly different by DMRT. P=0.05.

** Means collectively derived from all flushing position. 1.1 (youngest, 3 month-old), 1.2 (6 month-old) and 1.3 (oldest, 8 month-old).

Table 5. Fv/Fm values between temperature and storage period.

Temperature (℃)	Fv/Fm					
	0**	24	48	72	96	120
25	0.771 a	0.738 a	0.743 a	0.729 a	0.729 a	0.730 a
12	0.773 a	0 .661 bc	0.638 bc	0.599 c	0.602 bc	0.620 bc
9	0.773 a	0 .647 bc	0.652 bc	0.626 bc	0.599 c	0.598 c
6	0.770 a	0.655 bc	0.659 bc	0.636 bc	0.615 bc	0.623 bc
3	0.775 a	0.632 bc	0.637 bc	0.618 bc	0.604 bc	0.618 bc
1	0.770 a	0.601 bc	0.643 bc	0.632 bc	0.620 bc	0.631bc
-3	0.774 a	0.570 cd	0.580 cd	0.541 cd	0.507 d	0.470 d

* Means with the same letter are not significantly different by DMRT. P=0.05.

** Storage period (hrs).

*** Means collectively derived from the four mango cultivars 'Haden', 'Irwin', 'Mahachanok' and 'Jin Huang'.

Discussions

The exposition of plants to temperatures which induce chilling injury causes a fast decrease in the chlorophyll fluorescence value (Van Hasselt *et al*., 1983). A significant decrease in Fv/Fm values for all mango cultivars - 'Haden', 'Irwin', 'Mahachanok' and 'Jin Huang', was observed after 24 hours (Table 5) of incubation at 12^{o}C (Table 1; Table 4). This agrees with the onset of chilling injury in crops of tropical origin, wherein at 12^{o}C membrane changes and metabolic imbalances are initiated. 'Jin Huang', 'Haden', 'Irwin', and 'Mahachanok' showed the highest to lowest Fv/Fm values according to interactions between temperature and cultivar.

The Changes in Fv/Fm between the mango cultivars relative to flushing position (Table 3) indicated that the older leaves in 'Jin Huang' at positions 1.2 (0.724) and 1.3 (0.709) had higher Fv/Fm values after imposed chilling stress. However for 'Mahachanok', the older leaves 1.2 (0.566) and 1.3 (0.557) showed the opposite values upon low temperature stress. The leaf age and thicker epicuticular wax in old mango leaves (Urban and Jannoyer, 2004) contribute to the differences in

changes in Fv/Fm values. Kuk and Shin (2007) showed that varying leaf ages in cucumber leaves indicated developmental controls in the induction of plant defense and its ability to generate a stronger antioxidant sytem.

Fv/Fm values that were higher than 0.6 were considered as chilling tolerant and values starting from 0.5 and below as chilling sensitive. Applying this standard, 'Jin Huang', 'Haden' and 'Irwin' are chilling tolerant and 'Mahachanok' as chilling sensitive. The geographic origin of the mango cultivar influence the resulting changes in fluorescence yields. The genotype or the parental lines of mango cultivars transfers traits that will be intensified upon exposure to low temperature stress, such as better ROS scavenging capacity or higher unsaturation of fatty acids (Lyons *et al*., 1964).

Finally, decline in Fv/Fm values were attributed to a rise in stomatal limitation of net CO_2 assimilation rate and a decrease in Rubisco activity (Allen *et al*., 2000) as observed in potted mango plants ('Tommy Atkins' x 'Turpentine'). Leaf detachment acts by hastening the decrease of chlorophyll as reported by Potvin (1985) while studying its effects on bean, maize and cucumber. With simultaneous water loss or reduced water potential, rapidly decreases the Fv/Fm (Potvin, 1985). Lastly, better carbohydrate turnover capacity was associated to chilling tolerance in tolerant maize genotypes (Fracheboud *et al*., 1999). Such that, upon resumption to optimum temperatures the demand for energy was sufficiently supplied.

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利用葉綠素螢光反應偵測芒果(*Mangifera indica* L.)葉片對低溫的敏感性

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關鍵字：寒害逆境、芒果、葉綠素螢光

摘要：本試驗欲了解'Haden'、'Irwin'、'Mahachanok'及 'Jin Huang' 等芒果品種於寒害溫度下之反應。將切取的芒果葉片利用非破壞性的葉綠素螢光測定其在低溫逆境下之變化，並在寒害溫度下比較 Fv/Fm 值的變化。葉片放置於七種不同溫度下，以25℃做為對照組，接著寒害溫度為 12℃、9℃、6℃、3℃、1℃及凍害溫度-3℃。結果顯示，芒果葉片在寒害溫度 12℃下 24 小時，Fv/Fm 會明顯下降。在低溫敏感品種之 Fv/Fm 值，會隨著溫度降低而下降，但在低溫耐受品種之 Fv/Fm 值並不因低溫逆境而影響。Fv/Fm 值在 0.6 以上可歸類為寒害耐受品種，而 0.5 以下者為寒害敏感品種。結果顯示'Jin Huang'、'Haden'、'Irwin'對低溫忍受性較高，而'Mahachanok'則對寒害較敏感。

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