# Indices for Chilling Tolerance of 'Irwin' Mango Leaves (*Mangifera indica* L.)

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Key words: Mango, Low temperature stress, Chlorophyll fluorescence, Electrolyte leakage, Ethylene production, Respiration

## Summary

The objective of the research was to determinate of low temperature stress response in 'Irwin' mango leaves by using chlorophyll fluorescence, electrolyte leakage, ethylene production and respiration. 'Irwin' mango leaves were exposed to 25, 12, 9, 5, 3, 1 and -3$^{o}$C for determination the changes in chlorophyll fluorescence and to 25, 9, 1 and -3$^{o}$C for determination the changes in electrolyte leakage, ethylene production and respiration. Results showed that chlorophyll fluorescence tended to decrease at prolonged exposure to low temperatures, while electrolyte leakage increased as temperature decreased and duration increased. The changes under low temperature stress in ethylene production and respiration were not clear. Out of the four methods used for the screening of chilling tolerance in the leaves of mango cultivars, using chlorophyll fluorescence was the most effective.

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## Introduction

Many plants, especially those native to warm habitats, are injured when exposed to low nonfreezing temperatures (Lynch, 1990). Chilling tolerance is the ability of a plant to tolerate low temperatures (0–15$^{o}$C) without injury or damage (Somerville, 1995), while cold acclimation is an enhanced tolerance to the physical and physiochemical vagaries of freezing stress (Guy, 1990; Thomashow, 1999). Both cold acclimation and chilling tolerance involve an array of biochemical, molecular and metabolic processes (Thomashow, 1999; Larkindale *et al*., 2005; Kotak *et al*., 2007; Zhu *et al*., 2007).

Exposure of plants to low temperature stress leads to the modification of metabolism. Low temperature stress changes the structure, catalytic properties and function of enzymes (Kubien *et al*., 2003) and membrane metabolite transporters. Interestingly, regulatory mechanisms of plants become active and function to restore normal metabolite levels, and most importantly, metabolic fluxes (Schwender *et al*., 2004).

Many methods have been employed for evaluating low temperature stress of plants. Chlorophyll fluorescence (Rose and Haase 2002), electrolyte leakage (Mancuso *et al*.,2004), changes in ethylene production (Arshad and Frankenberger, 2002) and respiration (Nilsen and Orcutt, 1996) are among the many physiological indicators used for conifers.

Chlorophyll fluorescence can be used as a rapid, sensitive and noninvasive probe in the evaluation of plant performance and detect stress-induced perturbations in the photosynthetic apparatus (Baker, 2008). Any stress applied to green plant tissue which directly or indirectly affects photosynthetic metabolism is likely to change the yield (i.e. Fv/Fm) (Smillie and Hetherington, 1983).

Electrolyte are contained within the membranes of plant cells. These membranes are sensitive to environmental stresses. In plant membranes, these changes are often associated with increases in permeability and loss of integrity (Campos *et al*., 2003). Unstressed, undamaged plant cells maintain electrolytes within the membrane. As the cells are subjected to stress, electrolytes leak into surrounding tissues. An estimation of cell damage and hardiness can be made by comparing the conductivity of the leaked contents from injured and uninjured tissues in water (Mattsson, 1996).

Ethylene production by plants increases as a result of environmental stress or wounding, and measurement of stress ethylene can be a useful indicator of the onset of stress and/or the degree of

stress (Harber and Fuchigami, 2000). Temperature stress changes ethylene production differently in different plant species (Arshad and Frankenberger, 2002).

The respiration rate of plant under temperature stress are also differently in different plant species. Lyons and Riaison (1970) explain the effect of chilling on respiration associated with direct injury. Arrhenius plots of the respiration rate of mitochondria from chilling sensitive plants (tomato and cucumber fruit, sweet potato root) show a linear drop from 25$^{o}$C down to 9-12$^{o}$C. From this point to 1.5$^{o}$C the slope increased. Chilling resistant plants (cauliflower buds, potato tubers, beet root), on the other hand, showed a linear decrease over the entire temperature range. It has been report that increase in respiration occurred at the onset of injury and the decrease on death (Lewis and Morris, 1956).

The objectives of this study want to determination of low temperature stress in 'Irwin' mango leaves by using chlorophyll fluorescence, electrolyte leakage, ethylene production and respiration.

## Materials and Methods

**Plant Materials**

Mature 'Irwin' mango (*Mangifera indica* L.) leaves were collected at the Horticultural Research Station, College of Agriculture and Natural Resources, National Chung Hsing University in Taichung county, Taiwan. Leaves were collected and placed in polyethylene bags that were labeled accordingly and immediately transported back to the postharvest laboratory, National Chung Hsing University for further analysis within 1 h.

**Chlorophyll Fluorescence**

Detached leaves were rinsed 1 time with water to remove surface dirt on mango leaves and were leaving approximately 5 cm. length of the middle portion. The middle portion of the leaf was redundant and 1 cm. guide circle was made on parallel side in between the midvein so that successive measurements shall be collected on the same part of each leaf (Smillie and Hetherington, 1983). The middle of leaf was placed at the center of plastic petri dishes. In order to minimize water loss plastic petri dishes 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, 5, 3, 1 and -3$^{o}$C) and incubated in a low temperature incubator in the dark with daily measurements beginning at 0 h and subsequently at 24, 48, 72 and 96 h.

**Electrolyte leakage**

Mango leaves were cut into discs with 1.1 cm. in diameter and rinsed with pure water to eliminate the external residues. Discs were placed in the plastic petri dishes. In order to minimize water loss plastic petri dishes 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, 5, 3, 1 and -3$^{o}$C) and incubated in a low temperature incubator in the dark for 72 h. Every 24 h interval, three discs were removed and put in the test tubes and added with 5 ml pure water and then shaken the test tube for 3 h. The conductivity of the solution ($E_1$) was read with a conductivity meter. After that, the test tubes were frozen for 24 h in order to kill the tissue. After freezing, the test tubes were shaken for 3 h and the conductivity of this solution ($E_2$) was recorded. The percentage of electrolytes was calculated as follows: % Electrolyte = $E_1/E_2$ x 100

**Ethylene production**

Detached leaves were rinsed 1 time with water to remove surface dirt on mango leaf and cut into discs with 1.1 cm. in diameter. Discs were placed in the plastic petri dishes. In order to minimize water loss plastic petri dishes were lined with two layers of tissue paper and added with 3 ml pure water. Leaf samples were subjected to six temperature treatments (25, 12, 9, 5, 3, 1 and -3$^{o}$C) and incubated in a low temperature incubator in the dark with daily measurements beginning at 0 h and subsequently 24, 48, 72, and 96 h. Discs were removed and put in 25 ml Erlenmeyer flasks and enclosed with serum caps. The Erlenmeyer flasks were stored for 24 h at different temperature treatments, then 1 ml gas samples were collected by syringe for determination of ethylene production with a gas chromatograph (Shimadzu. FID.8A.).

**Respiration**

Respiration was measured as $CO_2$ production. Detached leaves were rinsed 1 time with water to remove surface dirt on mango leaf and cut in discs with 1.1 cm. in diameter. Discs were placed in the plastic petri dishes. In order to minimize water loss plastic petri dishes were lined with two layers of tissue paper and added with 3 ml pure water. Leaf samples were subjected to six temperature treatments (25, 12, 9, 5, 3, 1 and -3$^{o}$C) and incubated in a low temperature incubator in the dark with daily measurements beginning at 0 h and subsequently 24, 48, 72, and 96 h. Discs were removed and put in 25 ml Erlenmeyer flasks and enclosed with serum caps. The Erlenmeyer flasks were stored for 24 h at different temperature treatments, then 1 ml gas samples were collected by syringe for determination of $CO_2$ production with an infrared gas analyzer (Maihak. UNOR 610).

**Statistical analysis**

Data were subjected to analysis of variance using the general linear model of Costat appropriate for a repeated measures experiment. Treatment means were distinguished using a Duncan's Multiple Range Test. Significant differences were established at the $P<0.05$ level of probability.

## Results and Discussion

**Chlorophyll fluorescence of 'Irwin' mango leaves**

Chlorophyll fluorescence has been used to quantify chill-induced photoinhibition to leaves. Chilling stress damage to leaves may be determined by measuring the decrease in the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) (Oquist and Wass, 1988). In the present study, 'Irwin' mango leaves were determined the changes in chlorophyll fluorescence to low temperature (Fig. 1).

The seven temperature levels were compared for the effect on chlorophyll fluorescence. The Fv/Fm values were remained higher at 25$^{o}$C during exposure time. Whereas the Fv/Fm values continue to decreased with a further drop in temperature, which the lowest values of Fv/Fm were occurred at freezing temperature (-3$^{o}$C). This proves that the photosynthetic capacity was affected with the sustained exposure to low temperature (Whiley *et al.*, 1997).

The inherent sensitivity of the plant to low temperature is dependent on the time course (i.e. duration under low temperature) and extent (i.e. degree of low temperature). After 24 h of storage at low temperature, the Fv/Fm values showed a significant drop and there after showed a decreasing trend to decrease. Recovery is species dependent relative to its inherent sensitivity, cold duration and changes in chloroplast ultrastructure (Kratsch and Wise, 2000).

**Electrolyte leakage of 'Irwin' mango leaves**

Chilling impairments mainly consist of alteration of metabolic processes, decreased in enzymatic activities, reduction of photosynthetic capacity and changes in membrane fluidity among others (Dubey, 1997). Such changes are frequently related to an increase in membrane permeability, affecting membrane integrity and cell compartmentation under stress conditions. Increased rates of solute and electrolyte leakage occur in a variety of chilled tissues and have been used to evaluate membrane damage following chilling (Campos *et al.*, 2003).

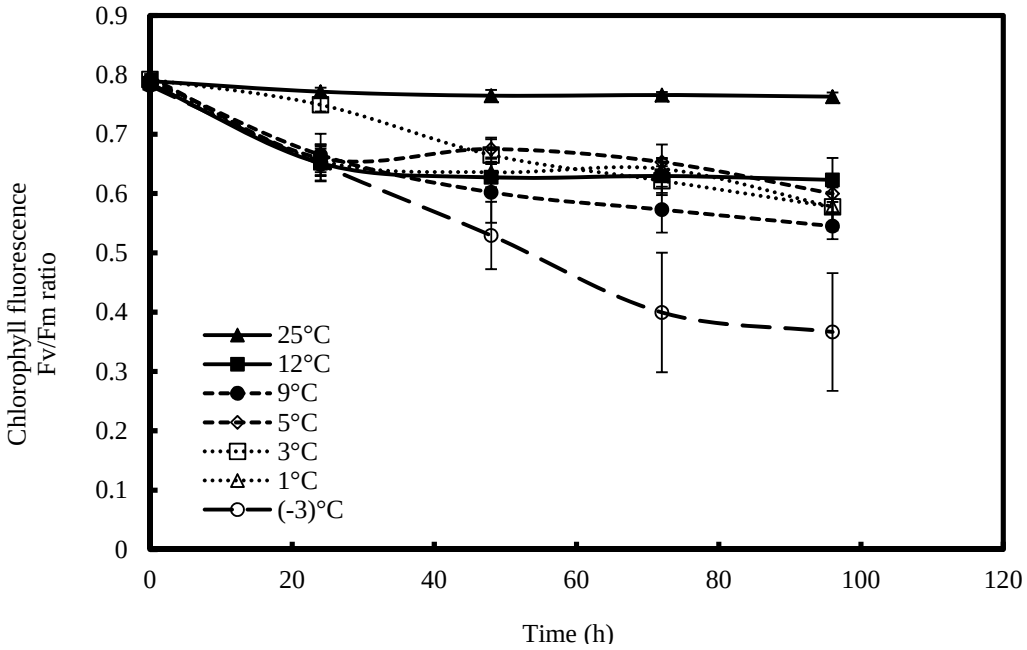


Figure 1. Effect of low temperature treatments on chlorophyll fluorescence (Fv/Fm) in 'Irwin' mango leaves.

The increase in electrolyte leakage of 'Irwin' mango leaves is depending on the level of temperature and duration (Fig. 2). The four temperature levels showed a significant different in electrolyte leakage. A strongly increase in electrolyte leakage were occurred at the freezing temperature (-3$^{o}$C), whereas 1, 9 and 25$^{o}$C, the electrolyte leakage had a slightly increase during periods. The increases in electrolyte leakage when the temperature was reduced or the duration increase as an indication of membrane damage. Saltveit (2002) reported for tomato a progressive increase in electrolyte leakage over a few days of chilling, while kiwifruit showed an increase up to 15% when exposed at 2$^{o}$C for 40 h (Gerasopoulos *et al.*, 2006). Low-temperature breakdown, a disorder which causes considerable quality losses during prolonged cold storage in kiwifruit, appears to be related to factors affecting membrane function (Gerasopoulos *et al*., 2006).

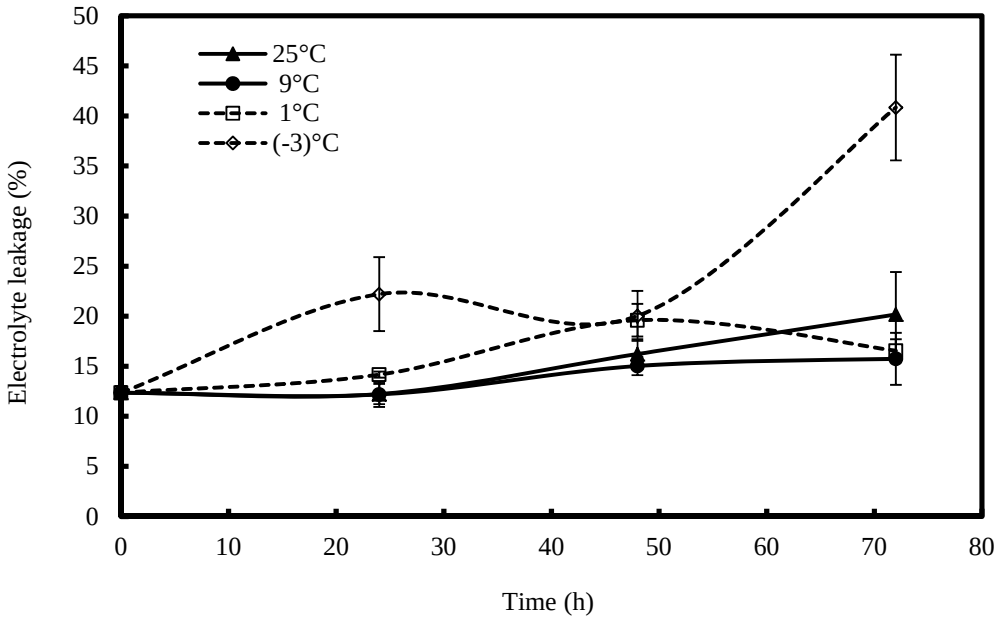


Figure 2**.** Effect of low temperature treatment on the electrolyte leakage in 'Irwin' mango leaves.

**Ethylene production of 'Irwin' mango leaves**

Ethylene is an endogenous plant hormone which plays important roles in plant growth and development such as seed germination, fruit ripening, senescence, and extension growth. Ethylene production is a common response to most stresses, including freezing, chilling, wounding, water, salt, and mineral stress (Harber and Fuchigami, 2000).

Ethylene production of 'Irwin' mango leaves was relatively high at 25$^{o}$C, whereas it was markedly decreased at 9, 1 and -3$^{o}$C (Fig. 3). Similar result was observed for wheat seedling (Tanino and McKerie, 1985) and oil palm seed (Corbineau *et al.,* 1990). The reduction of ethylene production at low temperature may associated with the conversation of ACC to ethylene was inhibit at low temperature (Ben-Amor *et al.,* 1999).

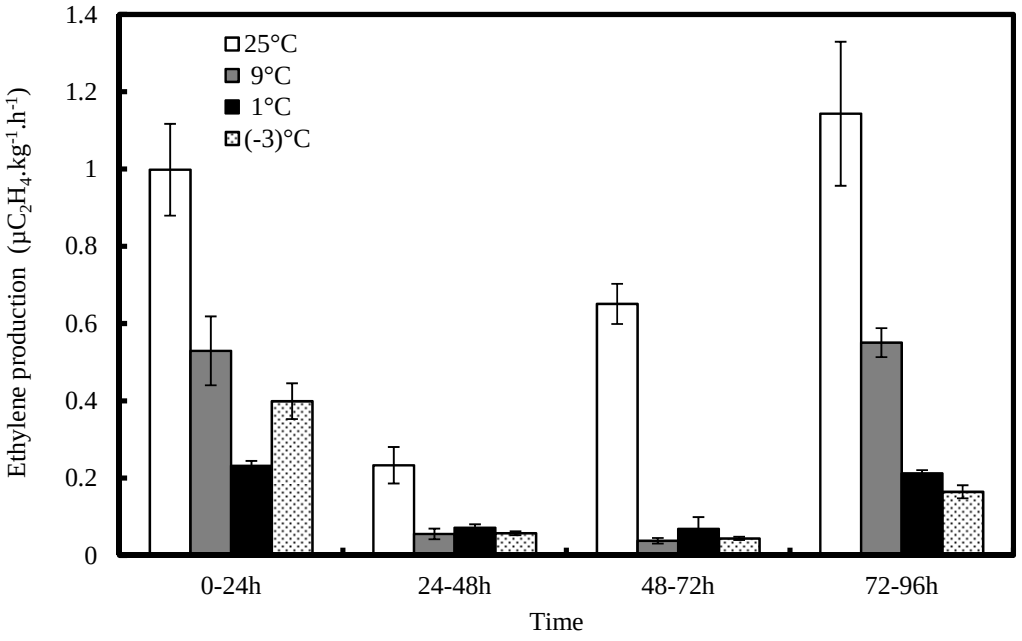


Figure 3. Ethylene production of 'Irwin' mango leaves exposed to low temperature.

**Respiration of 'Irwin' mango leaves**

The respiration peak of 'Irwin' mango leaves had a significant highest at 25$^{o}$C, followed by a dramatic decrease at 12$^{o}$C, which it is decrease more than 60% when compared with 25$^{o}$C. While at freezing temperature (-3$^{o}$C) showed the lowest (Fig. 4). The response of respiration to low temperature is depending on the species of plant and tissue organs. Some plants such as tomato (Kurets *et al.*, 2003), Arabidopsis (Talts *et al.*, 2004), mung bean and pea (Munro *et al.*, 2004) display a decrease in respiratory rate after exposure to low temperature, whereas some other species either show no decrease or even increase in respiration, as observed in maize leaves (Ribas-Carbo *et al.*, 2000) , and roots of *Plantago* (Covey-Crump *et al.*, 2002), rice and wheat (Kurimoto *et al.*, 2004).

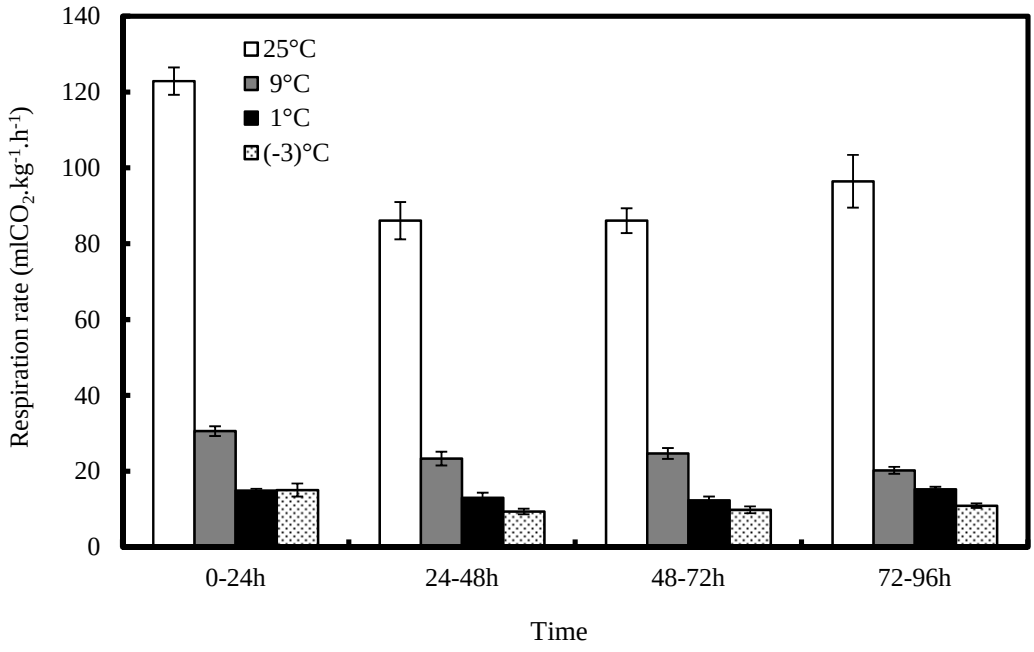


Figure 4. Respiration rate of 'Irwin' mango leaves exposed to low temperature.

## Conclusion

The responses of 'Irwin' mango leaves exposed to chilling temperature stress by using four methods (i.e. chlorophyll fluorescence, electrolyte leakage, ethylene production and respiration) in order to obtain suitable indexes. Chlorophyll fluorescence trend to decrease at low temperature and prolong periods, while electrolyte leakage increase at temperature decrease and duration increase. The changes in ethylene production and respiration under stress, the result showed no clear but all of both trend to decrease at low temperature. All four methods have been used to determine chilling damage in 'Irwin' mango leaves, but entail different problems. The ethylene and respiration are unclear when the plant exposure to chilling temperature. The electrolyte leakage measurements are less time consuming, but screening of larger populations of plant would still require considerable efforts. Chlorophyll fluorescence measurements are quick and more convenient than others.

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# '愛文'芒果耐低溫逆境篩選指標的建立

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關鍵字：芒果、低溫逆境、葉綠素螢光、離子滲漏率、乙烯生成率、呼吸率

**摘要：**本試驗使用'愛文'芒果葉片，將其置於低溫逆境下並測定其葉綠素螢光、離子滲漏率、乙烯生成率和呼吸率，以選擇最適之耐逆境指標。將'愛文'芒果葉片置於25、12、9、5、3、1、-3℃的低溫下，調查其葉綠素螢光之改變。然而僅取 25、9、1、-3℃等溫度測定電解質滲透率、乙烯生成量以及呼吸作用的改變。結果顯示，葉綠素螢光會隨著低溫處理時間增長而有下降的趨勢，而電解質滲漏率則會隨著溫度下降以及時間增長而上升。在低溫逆境下乙烯生成量與呼吸率的改變則不明顯。上述四種用檢測芒果不同品種葉片在高溫以及低溫下其耐受性的方法中，以葉綠素螢光法最有效率。

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