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BRIEF COMMUNICATION

Chilling stress and chilling tolerance of sweet potato as sensed by chlorophyll fluorescence

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Abstract

We studied changes in the chlorophyll (Chl) fluorescence components in chilling-stressed sweet potato (*Ipomoea batatas* L. Lam) cv. Tainung 57 (TN57, chilling-tolerant) and cv. Tainung 66 (TN66, chilling-susceptible). Plants under 12-h photoperiod and 400 μmol m^{-2} s^{-1} irradiance at 24/20 °C (day/night) were treated by a 5-d chilling period at 7/7 °C. Compared to TN66, TN57 exhibited a significantly greater basic Chl fluorescence (F_0), maximum fluorescence (F_m), maximum fluorescence yield during actinic irradiation (F_m'), and the quantum efficiency of electron transport through photosystem 2, PS2 (Φ_{PS2}). Chilling stress resulted in decrease in the potential efficiency of PS2 (F_v/F_m), Φ_{PS2}, non-photochemical fluorescence quenching (NPQ), non-photochemical quenching (q_N), and the occurrence of chilling injury in TN66. Chilling increased the likelihood of photoinhibition, characterized by a decline in the Chl fluorescence of both cultivars, and photoinhibition during low temperature stress generally occurred more rapidly in TN66.

Additional key words: cultivar differences; *Ipomoea*; photosystem 2; quantum yield.

Under natural conditions, photosynthesis is regulated biochemically to maintain a balance between the rates of its component processes and concentrations of metabolites in response to environment changes (Singsaas *et al.* 2000). Chloroplasts are the major target of many environmental stress factors. Plants respond to sudden and sustained fluctuations in irradiance and high and low temperatures *via* their chloroplast molecular redox signalling transduction mechanisms that initiate and network to induce marked modulations in chloroplast components, ultimately leading to acclimation of the photosynthetic apparatus (Anderson *et al.* 1997). Plants adapt photosynthesis within a certain range to prevailing environment, and the sensitivity of photosynthesis to stress varies among plant species and cultivars. Chilling stress reduces the capacity of photosynthetic systems to utilize incident photons and leads to photoinhibition (Jung *et al.* 1998). Photoinhibition of photosynthesis is characterized by a reduction in the quantum yield of photochemistry and a decrease in chlorophyll (Chl) fluorescence. Photoinhibition entails not only the inhibition of photosystem 2 (PS2) but also increases thermal de-excitation of excited Chl (Demmig-Adams and Adams 1992). Photoinhibition of photosynthesis has been reported for chilling-susceptible plants under high irradiance at chilling temperatures of 0–15 °C. Furthermore, annual plants of temperate regions undergo photoinhibition in winter when they are exposed to moderate irradiance at chilling temperatures (Somersalo and Krause 1989). Several indicators support this assumption: periods of low temperature are accompanied by a lower Chl content, decreased activities of Calvin cycle enzymes, poor development of the chloroplasts, an increased pool size of xanthophyll pigments, reduced photosynthetic capacity, decreased quantum efficiency of PS2 and CO_2 fixation, and increased dissipation of excitation energy in the PS2 antennae and photo-damage to PS2 reaction centres, RCs (Andrews *et al.* 1995, Haldimann *et al.* 1996, Kingston-Smith *et al.* 1997, Fryer *et al.* 1998). The mechanisms involved differ, depending on species and cultivars, the degree of hardiness, irradiance, temperature, and the duration of the chilling stress (Guy 1990, Thomashow

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Abbreviations: Chl – chlorophyll; F – fluorescence; PS2 – photosystem 2; RC – reaction centre; ROS – reactive oxygen species; Φ_{PS2} – quantum efficiency of electron transport through photosystem 2.

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1999). In past years, Chl fluorescence measurements were commonly used to study the responses of plants to low temperatures, including the functioning of the photosynthetic apparatus in maize (Massacci *et al.* 1995, Janda *et al.* 1998, Kościelniak and Biesaga-Kościelniak 2006), rice (Sthapit *et al.* 1995), bean (Guy *et al.* 1997, Melkonian *et al.* 2004), potato (Havaux and Davaud 1994), barley (Król *et al.* 1999), wheat (Öquist *et al.* 1993), spinach (Schöner and Krause 1990, van Wijk and van Hasselt 1993), and tomato (Jung *et al.* 1998) both in controlled environments and in the field under different chilling. Moreover, Chl fluorescence measurements have been used in screening for cold tolerance in cultivars (Dolstra *et al.* 1994, Ribas-Carbo *et al.* 2000).

However, the Chl fluorescence of sweet potato affected by low temperatures has not yet been examined and this is why we compared two cultivars grown in Taiwan where low temperatures and low irradiances are the primary physiological constraints to production in winter. Two cultivars of sweet potato Tainung 66 (TN66) and Tainung 57 (TN57) differently respond to chilling (Pai *et al.* 2004): TN57 shows greater chilling tolerance than TN66 in terms of growth rates, leaf water content, ion leakage, and anti-oxidative systems. TN57 is one of the most widely grown sweet potato cvs. in Taiwan due to its high yields.

Cuttings of TN66 and TN57 plants about 0.3 m in length were planted in 0.13 m plastic pots containing a medium consisting of peat moss, loamy soil, and sand in a ratio of 2 : 1 : 1. Plants were planted in September 2004 in a greenhouse of Chinese Culture University. They were evenly spaced every 0.5 m to encourage similar growth rates and sizes. Plants were watered with half-strength Hoagland solution every other day to maintain optimal irrigation and were allowed to grow for a month before the low temperature stress was imposed. During the period of plant growth, average day/night temperatures were 30/22 °C and average day length was 12 h. Plants were randomly placed in a growth chamber for a week-long acclimation under a 12-h photoperiod with an irradiance of 400 μmol m^{-2} s^{-1} at 24/20 °C and a relative humidity (RH) of 80 %. The plants were then exposed to chilling at 7/7 °C for 5 d with a 12-h photoperiod, 400 μmol m^{-2} s^{-1} irradiance, and 80 % RH. The experiment was independently performed twice for a randomized design of the growth environment, sampling day, and Chl fluorescence analysis.

Chl fluorescence parameters were quantified with a portable modulated fluorometer (Mini-Pam Photosynthesis Yield Analyzer, *Walz*, Effeltrich, Germany) in three plants from each period of time. All measurements were performed on a mature, healthy, fully expanded third leaf of the plants (Fracheboud *et al.* 1999) at 0, 3, 12, 24, 72, 96, and 120 h of the chilling period. The plants were dark-adapted for 40 min prior to the measurements. After clamping a leaf-clip holder (*2030-B*, *Walz*) onto the leaf, the basic or minimum (dark) fluorescence, F_0, was obtained upon excitation of leaves with a weak beam. The samples were then irradiated with "white actinic light" of 55 μmol m^{-2} s^{-1} in the growth chamber. The value of fluorescence (F_s) was thereafter recorded. A second saturating pulse at 6 000 μmol m^{-2} s^{-1} was imposed to determine the maximum fluorescence level in light-adapted leaves (F_m). Measurements of F_m and F_m' were performed with the measuring beam automatically switching to 29 kHz during the saturation flash. The yield of variable fluorescence (F_v) was calculated as $F_m - F_0$. The ratio of F_v/F_m was used as a measure of the maximum photochemical efficiency of PS2 (Butler 1978). The quantum yield of electron transfer at PS2 (Φ_{PS2}) is a measure of the overall efficiency of the PS2 reaction centres (RCs) in light. From these data, several parameters can be computed based on the modulated fluorescence kinetics: NPQ = $(F_m - F_m')/F_m'$; $q_P = (F_m' - F_s)/(F_m' - F_0')$; $q_N = (F_m - F_m')/(F_m - F_0)$; and $\Phi_{PS2} = (F_m' - F_s)/F_m'$ (Schreiber *et al.* 1986, Van Kooten and Snel 1990, Krall and Edwards 1992, Ghashghaie and Cornic 1994). Statistical analyses with unpaired *t*-test were made using *SAS8.2* (SAS Institute, Cary, NC, USA).

When TN57 plants were placed in a growth chamber for 5 consecutive days at 7 °C, F_0 in TN57 increased from 0 to 96 h, and then dropped again (Fig. 1*A*). In TN66 the F_0 slightly decreased till 48 h, and then slightly increased. TN57 showed a significantly higher F_0 than TN66 at all times except for at 120 h. A gradual decrease in the F_m value was observed for both cvs. during the time of chilling (Fig. 1*B*), with the exception of elevation from 96 to 120 h for TN66. Thus chilling stress caused a decrease in F_m of both TN66 and TN57 plants. A slight continuous decrease was found in F_v/F_m for both cultivars (Fig. 1*C*), without significant differences. In healthy leaves, the F_v/F_m was close to 0.8, a value typical for uninhibited plants. A lower value indicates that a portion of the PS2 RCs was damaged (Somersalo *et al.* 1998, Jung *et al.* 1998).

The trends of F_m' and Φ_{PS2} for both cvs. were more or less parallel (Fig. 1*D,E*), the values from 12 to 96 h being significantly higher for TN57. In general, when plants were chilled for 5 d, they gradually lost their PS2 function as time went by. NPQ declined with low temperature treatment (Fig. 1*F*): TN66 had a significantly higher NPQ than TN57. This indicated thermal energy dissipation in the antennae. After 12-h chilling we did not find significant changes in q_N in both cvs. (Fig. 1*G*); this indicated that chilling accelerated photoinhibition. The q_N values were higher for TN66 than for TN57. Cultivars induced by chilling stress may be involved in imparting chilling tolerance.

A decrease in q_P was observed in both cvs. between 0 and 48 h, followed by an increase to a maximum at 120 h of chilling (Fig. 1*H*) The q_P did not show significant difference between cultivars in any time period.

Growth at a low temperature led to an obvious decrease in the indicated Chl fluorescence parameters,

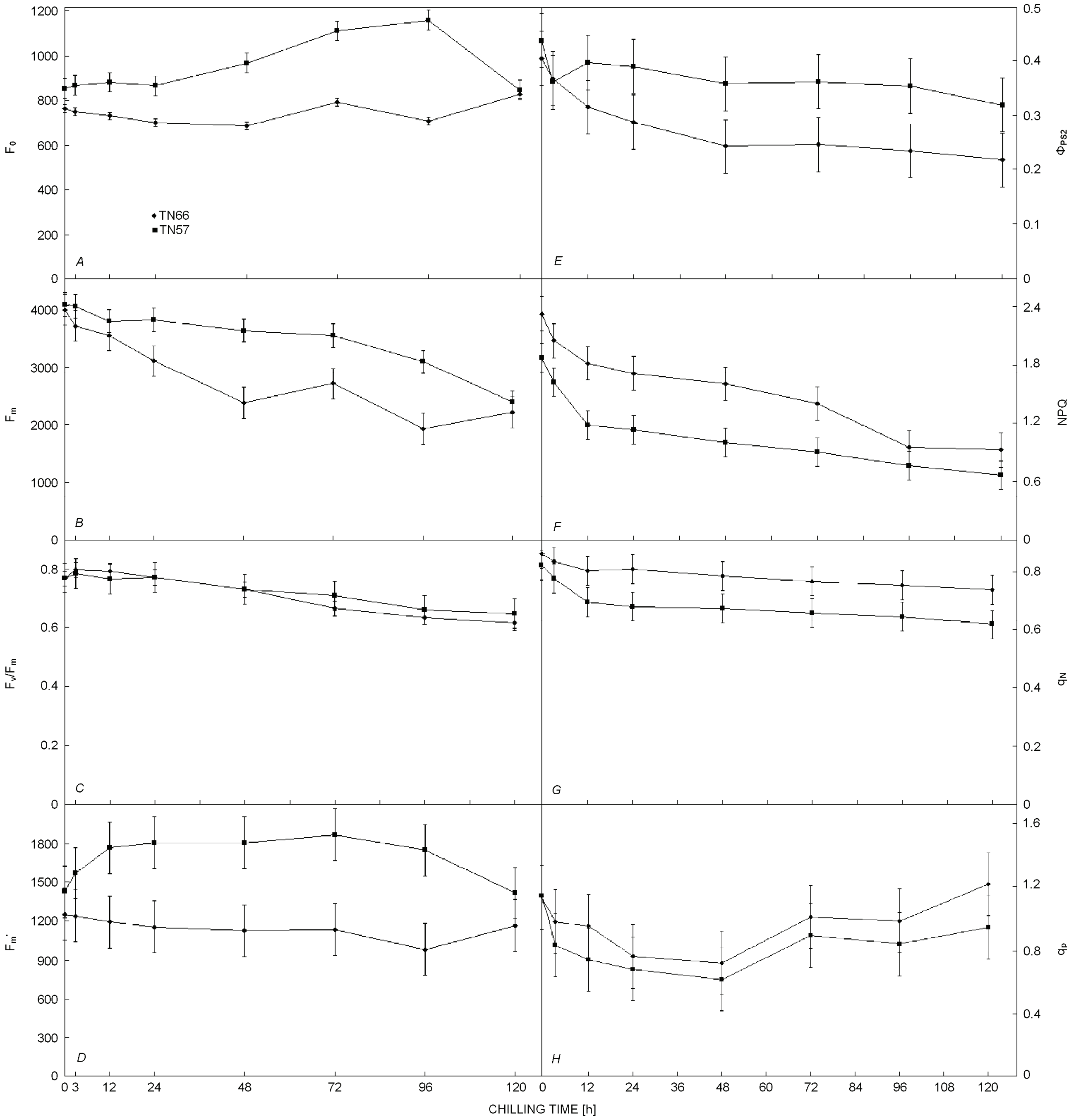

Fig. 1. Effects of 5 d of chilling at 7 °C (day and night) on the (*A*) F_0, (*B*) F_m, (*C*) F_v/F_m, (*D*) F_m’, (*E*) Φ_{PS2}, (*F*) NPQ, (*G*) q_N, and (*H*) q_P values of the chilling sensitive cultivar Tainung 66 (TN66) and the chilling tolerant cultivar Tainung 57 (TN57) of sweet potato. *Vertical bars* represent standard errors.

showing chronic photoinhibition. Exceptionally, F_0 (Fig. 1*A*), F_m’ (Fig. 1*D*), and q_P (Fig. 1*H*) gradually increased at different time periods, which may indicate non-radiative (thermal) energy dissipation. The thermal dissipation process is called non-photochemical quenching (q_N or NPQ), referring to the fact that the thermal dissipation of Chl's excited states competes with fluorescence emission as well as with photochemistry (*i.e.* photosynthesis). The decreases in NPQ or q_N are associated with a decrease in non-photochemical quenching. PS2 activity may regulate the response of photosynthesis to temperature changes. We found that the parameters Φ_{PS2}, F_m, F_v/F_m, NPQ, and q_N were characteristic of chilling stress.

F_0, F_m, F_m’, and Φ_{PS2} for TN57 were significantly higher than those of TN66 after different durations of chilling. The greater capacity of TN57 to maintain these quotients higher than in TN66 does not mean that F_v/F_m,

q_P, q_N, and NPQ of TN57 were superior under the same environment. These results suggest that the Chl fluorescence parameters were low-temperature-specific and were not expressed solely in response to increasing excess of photon energy. Chloroplast development in TN66 may be particularly sensitive to low temperatures, or alternatively, the pigments of the sensitive cv. might have been destroyed because of a high sensitivity to oxidative stress induced by the low temperatures. Protection mechanisms are present in both TN66 and TN57 which should prevent the leaves from experiencing excessive reductions in PS2 acceptors. In short, these higher Chl fluorescence values under chilling which help TN57 tolerate chilling could be used to select for the maintenance of photosynthetic capacity in plants bred for low temperatures.

We found that the lower leaves of all plants looked epinastic and senescent after 5 d of chilling. However, the leaves of TN57 plants appeared healthier and greener throughout the duration of the experiment (photos not shown). The tolerant cv. had higher rates of photosynthesis than the susceptible one, when subjected to chilling. The tolerant cv. may also benefit from warmer temperatures after a prolonged period of low temperatures. This hypothesis is in agreement with our observation that TN57 leaves recovered faster. Some of the damage to TN66 was irreversible once chilling damage had occurred (photos not shown). Recovery may reflect the repair capacity of the plants. Both cvs. behaved similarly when the leaves were developed at 24/20 °C, and hence the genotypic differences might be related to adaptation mechanisms induced by low temperatures.

In summary, Chl fluorescence components were used to indirectly measure the different functional levels of photosynthesis. We showed that the cultivars responded differently to chilling stress due to the different photosynthetic apparatus. F_m, F_v/F_m, Φ_{PS2}, NPQ, and q_N were characterized by a decreasing trend on different days of chilling. F_m and Φ_{PS2} of TN57 appeared tolerant to chilling-induced photoinhibition, and may be useful criteria for differentiating between plants. The average time required to measure the F_0 and F_m from a pre-dark-adapted sample was only 1 s. This means that many hundreds of individual plants may be screened per day, providing the scope for discovery of individuals that exhibit great tolerance to chilling stress.

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