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CHILLING SENSITIVITY OF CUCUMBER PLANTS MONITORED IN TERMS OF CHLOROPHYLL FLUORESCENCE

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Abstract — For three cultivars of chilling-sensitive cucumber plants, chilling sensitivity was evaluated in terms of photosynthetic activity using Chl fluorescence techniques. Low-temperature treatment caused a decrease in photosynthetic activities of cucumber leaves, measured as CO_2 exchange, as well as the decrease in the stomatal conductance. F_R of the three cultivars decreased after chilling for 24 h in light and the extent of decline of F_R was the greatest in 'Chosaeng' cultivar. When these plants were recovered from light-chilling, 'Chosaeng' and 'Samchuk' cultivars did not fully restore the original value of F_R after 24 h of recovery, in contrast to 'Ilmi' cultivar which showed a rather efficient recovery. The results of F_R study showed that 'Chosaeng' was most susceptible, whereas Ilmi was most resistant, to chilling among the three cultivars of cucumber plants. When quenching coefficients for chlorophyll fluorescence was analyzed after chilling the cucumber plants for 24 h in light, 'Chosaeng' elicited more rapid declines in the coefficients for photochemical quenching (qQ), non-photochemical quenching (qNP) and energy-dependent quenching (qE) than 'Ilmi' and 'Samchuk'. The implications of these observations are discussed in relation to the growth habits of the respective cultivars in the field. The results showed that measurement of chlorophyll fluorescence was an effective means of screening chilling tolerance of cucumber plants. Furthermore, the study on the chlorophyll fluorescence induction and fluorescence quenching charactersitics showed that low temperature could accelerate inhibition of photosynthesis in chilling-sensitive plants, by limiting Calvin cycle activity and disrupting, in part, the energy dissipation mechanims of the photosystem II.

INTRODUCTION

Temperature limits the geographical distribution and productivity of plants. Most tropical and subtropical plants suffer severe damage when they are exposed to chilling temperature ranging 1-12°C.[1] This phenomenon is termed chilling injury, and these plants are called chilling-sensitive plants. Chilling sensitivity of plants is known to be closely correlated with the degree of fatty acid unsaturation of membrane lipids.[2-4]

Chilling sensitivity has been evaluated by various methods, including the measurement of growth at chilling temperataures,[5] electrolyte leakage rates,[6] amounts of phosphatidylglycerol species in the plant membranes.[7] However, a method for assessing the level of stress injury should be rapid, sensitive and nondestructive to the plant tissue. Since Chl in the thylakoid membranes of chloroplasts emits a red fluorescence, of which, in part, variable fluorescence is responsive to changes in PS II activity,[8] Chl fluorescence measurements can be used to detect and quantify rapidly plant response to major environmental stresses.

Low temperature and irradiance are important examples of unfavorable environmental stresses on plants, and they have been identified as a potentially more serious cause of injury than chilling alone.[9] In many chilling-sensitive plants, exposure to low

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†*Abbreviations*: Chl, chlorophyll; F_m, maximal fluorescence; F_o, initial (dark level) fluorescence; F_R, maximal rate of rise of induced Chl fluorescence emission from dark-adapted leaves; F_v, variable fluorescence (F_m-F_o); F_v/F_m, fluorescence ratio; PS II, photosystem II; Q_A, primary electron acceptor of photosystem II; qE, energy-dependent quenching; qNP, non-photochemical quenching; qQ, photochemical quenching.

temperature under irradiation leads to pronounced functional inhibition of photosynthesis, whereas chilling in darkness results in little or no damage.[10] Therefore, evaluation for chilling sensitivity should not be based soley on tests for the cold resistance in the dark, but should also take into account sensitivity of the plants to chilling under irradiance and its capacity to recover from it.

The aim of the present study was to assess relative susceptibilities of the three cultivars of cucumber plants, which are most widely cultivated domestically, to low temperature-induced photoinhibition, and to obtain informations on mechanisms involved in the development of chilling damage to the photosynthetic machinery.

MATERIALS AND METHODS

Growth of plant materials and exposure to chilling. Three cultivars of cucumber plants (*Cucumis sativus* L.), namely, Ilmichungjang('Ilmi'), Pyunggangnaebyung samchuk ('Samchuk'), and Chosaengnakhap ('Chosaeng') were grown for two weeks in vermiculite at 25°C with 16 h of light daily (60 μmol m^{-2} s^{-1}). The primary leaves attached to the plants were used for experiments. For chilling treatment cucumber plants were transferred to a cold room (4°C) with continuous illumination of a light intensity with 60 μmol m^{-2} s^{-1}.

Measurement of CO_2 exchange rates and stomatal conductance. The primary leaf, still attached to the plant, was enclosed in a temperature-controlled assimilation chamber. The rate of light-saturated CO_2 exchange rate and leaf stomatal conductance was measured on the primary leaf at 25°C under ambient CO_2 conditions with a infrared gas analyzer system (LCA 2, ADC Co., U.K.). CO_2 exchange rate, stomatal conductance was calculated according to von Caemmerer and Farquhar (1981).[11]

In vivo chlorophyll fluorescence measurement. Room temperature Chl a fluorescence of leaves was measured after predarkening the plants with pulse modulation Chl fluorometer system (PAM 101-102; Walz, Effeltrich, Germany) according to Horton and Hague (1988).[12] The dark level fluorescence (F_o) was determined by a weak, modulated light of 1.6 μmol m^{-2} s^{-1}, maximal fluorescence (F_m) of a dark-adapted leaf by a saturating flash of strong white light of 4 x 10^3 μmol m^{-2} s^{-1} (PAR) of 1 s duration. Variable fluorescence (F_v) was induced by a nonmodulated white light (200 μmol m^{-2} s^{-1}) supplied by a Schott KL 1500 light source. To analyze quenching mechanisms, saturation pulses wer triggered every 30 s. The quotient, F_v/F_m, according to Butler (1978),[13] was used as a measure of the potential efficiency of PS II.[8]

Quenching characteristics of fluorescence induction were measured with a PAM 101 fluorometer according to Schreiber *et al.* (1986).[14]

RESULTS AND DISCUSSION

Changes in CO_2 uptake and stomatal conductance. Figure 1 shows time course of CO_2 uptake rates and stomatal conductance in 'Ilmi' cultivar of cucumber plants during treatment at 4°C with illumination at an intensity of 60 μmol m^{-2} s^{-1}. CO_2 uptake declined with time of chilling, and finally to the 23% level of the inital rate after 24 h of light-chilling. Such a depression of photosynthetic CO_2 exchange may occur by either stomatal closing or dysfunction of biochemical mechanisms of chloroplasts. For identifying the cause of decline in CO_2 uptake, we studied the stomatal conductance of cucumber leaves. It also decreased with time of light-chilling and showed 41% of the initial value after 24 h (Fig. 1). These results suggest that photosynthetic CO_2 fixation rate is affected at low temperature by the limitation of CO_2 supply, due to the low temperature-induced stomatal resistance. Therefore, it is reasonably assumed that the mechanisms involved in chilling-dependent decrease of photosynthesis should include stomatal closure, as well as other factors such as photoinhibition, membrane damage and impairment of dark reactions.[15]

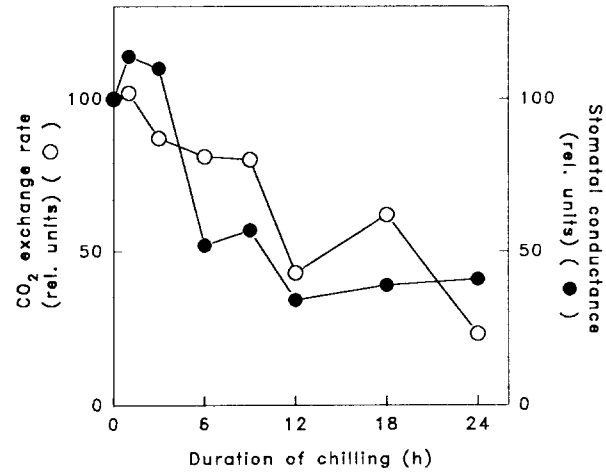

Figure 1. Inhibition of CO_2 exchange rates and leaf stomatal conductance during chilling of cucumber plants ('Ilmi'). 'Ilmi' cultivar of cucumber plants were treated at 4°C at a light intensity of 60 μmol m^{-2} s^{-1}. The initial values of light-saturated CO_2 exchange rate and stomatal conductance measured at 25°C were taken as 100% and corresponded to 7.5 μ mol CO_2 m^{-2} s^{-1} and 0.22 cm s^{-1}, respectively. The values were obtained from the results of two independent experiments. The deviation of values was within 5%. (○), CO_2 exchange rate; (●), stomatal conductance.

Inhibition and recovery of PS II activity. Chilling-induced changes in Chl fluorescence *in vivo* have been utilized to rapidly assess plant response to low, nonfreezing temperatures. For this purpose, we

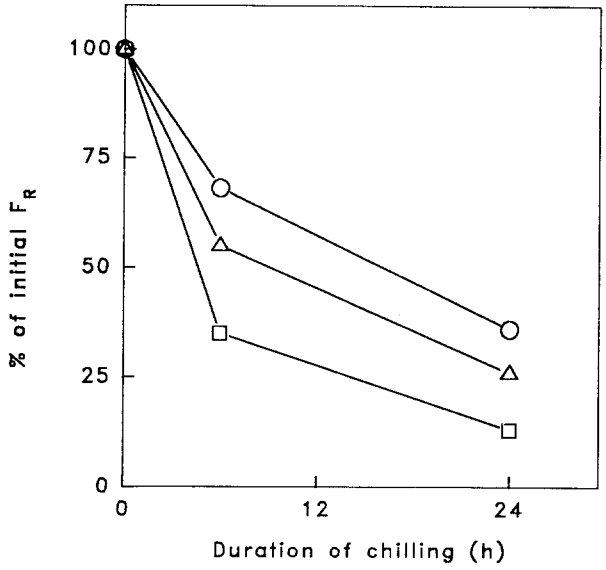

Figure 2. Effect of chilling on the F_R component of Chl fluorescence of cucumber plants. Cucumber plants were treated at 4°C in light at an intensity of 60 μmol m^{-2} s^{-1}. Then, these plants were predarkened for 20 min at 25°C before inducing Chl fluorescence. The initial values of F_R were taken as 100% and corresponded to 1.94, 2.5 and 1.95 in 'Ilmi', 'Samchuk' and 'Chosaeng', respectively. The values were obtained from the results of three independent experiments. The deviation of values was within 5%. (○), 'Ilmi', (△),'Samchuk' and (□), 'Chosaeng'.

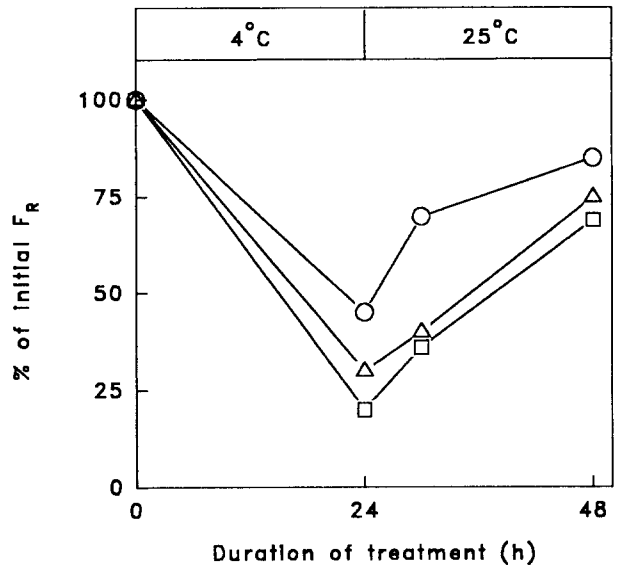

Figure 3. Effect of 24 h-chilling and subsequent recovery on the F_R component of Chl fluorescence of cucumber plants. Cucumber plants were treated at 4°C in light at an intensity of 60 μmol m^{-2} s^{-1}. Then, they were allowed to recover from the chilled state by incubating them at 25°C in light at the same intensity that had been used during chilling. The initial values of F_R were taken as 100% and corresponded to 1.94, 2.5 and 1.95 in 'Ilmi', 'Samchuk' and 'Chosaeng', respectively. The values were obtained from the results of three independent experiments. The deviation of values was within 5%. (○), 'Ilmi', (△), 'Samchuk' and (□), 'Chosaeng'.

employed F_R, which corresponded to the maximal rate of the induced rise of Chl fluorescence, as an index of the relative chilling sensitivity of plant tissue.[16]

Figure 2 shows the decline of F_R in the three cultivars of cucumber plants during treatment of light-chilling at 4°C. 'Chosaeng' showed the greatest susceptibility to low temperature among the three cultviars of cucumber plants, in contrast to 'Ilmi' that was relatively resistant, when their degrees of chilling sensitivity were determined in terms of F_R. This observation is consistent with the field-cultivation practices in our country, in that young plants of 'Ilmi' are usually grown in the early spring when the weather is still cool, whereas 'Chosaeng' seedlings are cultivated during summer when the air temperature is relatively high.

Figure 3 shows restoration of F_R at 25°C in the three cultivars of cucumber plants after light-chilling for 24 h at 4°C. Cucumber plants were first exposed to 4°C in light at an intensity of 60 μmol m^{-2} s^{-1} for 24 h to reduce the F_R down to a 20 to 40% level of the initial values. Then, restoration of F_R was followed by treating them at 25°C. The results showed that the three cultivars are clearly different in the extent of recovery of the photosynthetic appratus from low-temperature-induced inactivation. After recovery for 24 h under irradiation at an intensity of 60 μmol m^{-2} s^{-1}, 'Ilmi' reached 86% of the original value of F_R, whereas 'Chosaeng' and 'Samchuk' had reached only 70%.

Changes in quantum efficiency of PS II. A small degree of photoinhibition, measured as F_v/F_m (ratio of variable to maximal Chl fluorescence), was detected in leaves of plants exposed to the chilling treatment at 4°C for 24 h with illumination of light at an intensity of 60 μmol m^{-2} s^{-1} (Fig. 4A). The F_v/F_m ratio decreased from 0.78-0.82 to 0.77, 0.7, 0.65, after light-chilling of 'Ilmi', 'Samchuk', and 'Chosaeng', respectively. With the same treatment, F_o/F_m (ratio of initial to maximal Chl fluorescence) increased about 130%, 190% and 230%, with respect to the control in 'Ilmi', 'Samchuk', and 'Chosaeng', respectively (Fig. 4B).

Based on the general idea that F_v/F_m ratio indicates changes in quantum efficiency of photosynthesis during photoinhibition,[17] the lowering of F_v/F_m, that had been observed in 'Samchuk' and 'Chosaeng', signalizes depression of the potential quantum yield of the PS II at low temperature. As shown in Figure 4B, F_o increased strongly upon light-chilling of 'Samchuk' and 'Chosaeng'. This increase may contribute to the decreased F_v in those cultivars. Such a decrease in variable fluorescence, in turn, in

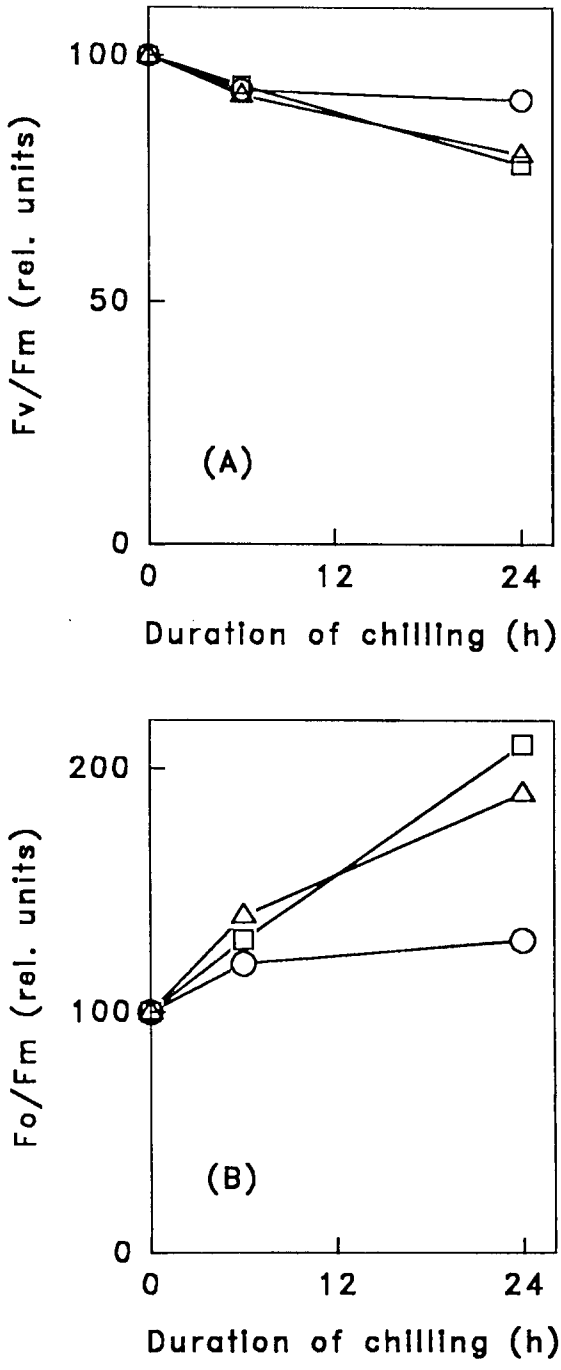

Figure 4. Changes in photosystem II activity, monitored in terms of the F_v/F_m (A) and the F_o/F_m ratios (B) of cucumber plants at chilling temperature. Cucumber plants were treated at 4°C in light at an intensity of 60 μmol m^{-2} s^{-1}. Then, these plants were predarkened for 20 min at 25°C before inducing Chl fluorescence. The initial values of F_v/F_m were taken as 100% and corresponded to 0.78, 0.82 and 0.83 in 'Ilmi', 'Samchuk' and 'Chosaeng', respectively. The initial values of F_o/F_m were taken as 100% and corresponded to 0.22, 0.18 and 0.17 in 'Ilmi', 'Samchuk' and 'Chosaeng', respectively. The values were obtained from the results of three independent experiments. The deviation of values was within 3%. (○), 'Ilmi',(△),'Samchuk' and (□), 'Chosaeng'.

'Samchuk' and 'Chosaeng' at low temperature could be attributed to a limitation of electron donation to PS II,[18] which might be caused by accumulation of the photosystem II reaction centers with stable reduced Q_A,[19] or a partial disconnection of the antennae from the centers.[20]

Changes in the coefficients for Chl fluorescence quenching. Figure 5A shows changes in the coefficients of photochemical quenching (qQ) in the three cultivars of cucumber plants which had been treated at 4°C for 24 h with illumination at an intensity of 60 μmol m^{-2} s^{-1}. Chilled plants were warmed to the room temperature and dark-adapated for 20 min prior to measuring Chl fluorescence. Then, the parameters of fluorescence quenching were determined from the recording traces of induced fluorescence signals from the leaves. The results showed that photochemical quenching (qQ) of 'Ilmi' remained farily constant with chilling, when compared with that of 'Samchuk' and 'Chosaeng', that showed a rapid decline. In particular, 'Chosaeng' almost completly lost qQ after light-chilling for 24 h. Since CO_2 is the final electron acceptor in the electron transport chain, an inhibition of CO_2 assimilation at low temperatures could be expected to affect the redox state of the photosystem II electron acceptor, Q_A, and thus photochemical quenching, qQ. With exposure to low temperature, there is an acculmulation of reduced Q_A, which in chilling-sensitive species is only slowly reoxidized and remains elevated even under steady state conditions.[21] Therefore, the decreased qQ component can be a consequence of limited dark reactions at chilling temperatures, since a limited activity of the Calvin cycle finally will result in accumulation of NADPH, and thus feedback limitation of electron transport may occur. The severe loss of qQ in 'Chosaeng' indicates that the operation of Calvin cycle in this cultivar is extremely sensitive to low temperature.

Figure 5B shows changes in the coefficients of non-photochemical quenching (qNP) in the three cultivars of cucumber plants which had been chilled for 24 h. 'Ilmi' showed a constant level of qNP during chilling. Contrastingly, 'Chosaeng' elicited a strong decline in qNP at the same treatment, while 'Samchuk' was intermediate between the two. The profiles for the coefficients for energy-dependent quenching (qE) changed in a comparable pattern to those of qNP during light-chilling of cucumber plants (Fig. 5C).

qNP can be contributed by several factors, such as energy-dependent quenching (qE), state transitions, and photoinhibition.[8] The major component of those factors is qE, which is believed to reflect a mechanism for energy dissipation at PS II. Moreover, qE is triggered by a rising proton concentration in the

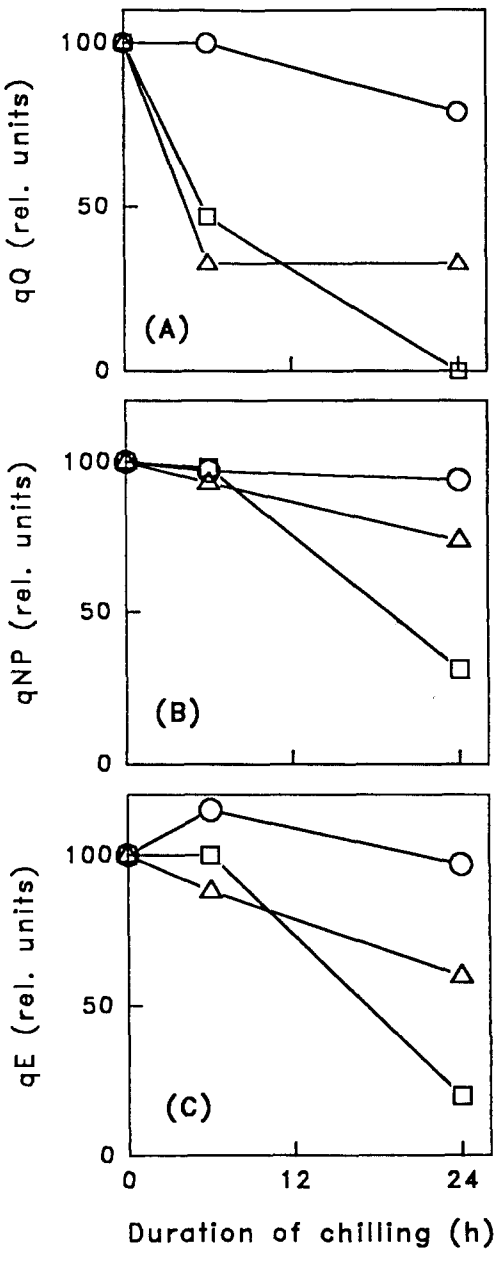

Figure 5. Effect of chilling on the Chl fluorescence quenching components qQ (A), qNP (B) and qE (C) of cucumber plants. Cucumber plants were treated at 4°C in light at an intensity of 60 μmol m^{-2} s^{-1}. Then, these plants were predarkened for 20 min at 25°C before inducing Chl fluorescence. The initial values of qQ were taken as 100% and corresponded to 0.24, 0.24 and 0.19 in 'Ilmi', 'Samchuk' and 'Chosaeng', respectively. The initial vales of qNP were taken as 100% and corresponded to 0.62, 0.57 and 0.58 in 'Ilmi', 'Samchuk' and 'Chosaeng', respectively. The initial vales of qE were also taken as 100% and corresponded to 0.34, 0.42 and 0.40 in 'Ilmi', 'Samchuk' and 'Chosaeng', respectively. The values were obtained from the results of three independent experiments. The deviation of values was within 5%. (○), 'Ilmi', (△), 'Samchuk' and (□), 'Chosaeng'.

thylakoid lumen.[22] Thus it is conceivable that low temperature induces rapid declines in qE and qNP, due to collpase of the build-up of proton concentration in the thylakoid lumen, which may occur due to limited electron transport. This declines in non-photochemical quenching enhances the loss of capacity to dissipate excess energy in a radiationless form. Therefore, the lowering of qNP and qE, such as that had been observed in 'Samchuk' and 'Chosaeng', indicates that these cultivars are more sensitive to deterioration of photosynthetic functions in response to chilling stress.

In summary, the use of room temperature chlorophyll fluorescence technique was found to be a useful tool in the determination of chilling sensitivity of cucumber plants. Moreover, the induction of fluorescence signals and analysis of fluorescence quenching characteristics suggested that low temperature-induced inhibition of photosynthesis is associated with limitation of Calvin cycle activity and with, in part, disruption of the energy dissipation mechanism of the PS II.

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