

## Effects of different nitrogen forms on photosynthetic rate and the chlorophyll fluorescence induction kinetics of flue-cured tobacco

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### Abstract

Net photosynthetic rate ( $P_N$ ) of tobacco plants grown with  $\text{NH}_4\text{-N}$  as the only N source was the lowest all the times, while  $P_N$  grown only with  $\text{NO}_3\text{-N}$  was the greatest until 22<sup>nd</sup> day, and  $P_N$  grown with both  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  (1 : 1) was the greatest. Maximal photochemical efficiency of photosystem 2 (PS2),  $F_v/F_m$ , and actual quantum yield of PS2 under actinic irradiation ( $\Phi_{PS2}$ ) in plants grown with only  $\text{NH}_4\text{-N}$  were greatest at early stage and then decreased and were smaller than those of other treatments. Photochemical quenching coefficient ( $q_P$ ) and non-photochemical quenching coefficient ( $q_{NP}$ ) in the  $\text{NH}_4\text{-N}$  plants were the greatest at all times. Hence excessive  $\text{NH}_4\text{-N}$  can decrease not only photochemical efficiency but also the efficiency of utilization of photon energy absorbed by pigments for photosynthesis. Therefore, excessive  $\text{NH}_4\text{-N}$  is a hindrance to photosynthesis of flue-cured tobacco. On the other hand, tobacco cultured with an appropriate mixture of  $\text{NO}_3\text{-N}$  with  $\text{NH}_4\text{-N}$  can sufficiently utilize photon energy and increase the efficiency of energy transformation.

*Additional key words:* actual quantum yield of PS2; *Nicotiana*; photochemical and non-photochemical quenching; photosystem 2.

Photosynthesis is a basic physiological process in crop production. In order to increase output, diversified cultivation measures are adopted to improve photosynthetic capability of crops (Dong *et al.* 1991). Nitrogen is the main constituent of proteins, chlorophyll (Chl), and enzymes involved in photosynthesis. Therefore, nitrogen affects photosynthesis of crops. The nitrogen absorbed by plants mostly includes  $\text{NO}_3\text{-N}$  and  $\text{NH}_4^+\text{-N}$ , and their uptake, deposition, and assimilation in crops are different. There have been many studies on the effects of N forms on photosynthesis, growth, yield, and quality of tobacco, but the relationship of nitrogen forms to energy conversion and distribution in photosynthesis has rarely been researched (Han 1996, Feng and Peng 1998, Yang *et al.* 1999). In order to provide a theoretical basis for the cultivation techniques of good quality and high yield tobacco, we studied the effects of N forms on Chl fluorescence and photosynthesis.

Tobacco seedlings (cv. K326) having 8 leaves were cultured in the nutrient solution [ $\text{g m}^{-3}$ ] N 40, P 20, K 80, Ca 80, Mg 10, Fe 1, Zn 0.1, Mn 0.1, B 0.05, Cu 0.01,

Mo 0.01 that was aerated every 24 h and exchanged every 7 d. Three treatments were included: (T1)  $\text{NO}_3\text{-N} : \text{NH}_4\text{-N} = 1 : 0$ ; (T2)  $\text{NO}_3\text{-N} : \text{NH}_4\text{-N} = 0 : 1$ ; (T3)  $\text{NO}_3\text{-N} : \text{NH}_4\text{-N} = 1 : 1$ . Net photosynthetic rate ( $P_N$ ) was measured with a portable photosynthesis system (LI-6400, LI-COR, USA) and Chl fluorescence was determined with a FMS2 fluorescence monitor (Hansatech, UK) between 09:30 and 10:30 after 16, 18, 20, 22, and 24 d. Seedlings were dark-adapted for 15 min before measurements. Four tobacco seedlings were measured in every treatment, and every tobacco seedling was measured three times. The room temperature was 18–22 °C. The measured fluorescence parameters were  $F_0$  (original fluorescence),  $F_v$  (variable fluorescence),  $F_m$  (maximal fluorescence) =  $F_v + F_0$ ,  $F_s$  (steady fluorescence),  $F_0'$  (original fluorescence after light adaptation), and  $F_m'$  (maximal fluorescence after light adaptation) =  $F_0' + F_v'$ .  $\Phi_{PS2}$ ,  $q_P$ , and  $q_{NP}$  were calculated as  $\Phi_{PS2} = (F_m' - F_s)/F_m'$ ,  $q_P = (F_m' - F_s)/(F_m' - F_0)$ ,  $q_{NP} = (F_m - F_m')/F_m'$ .

$F_v/F_m$  is directly related with the activity of photosynthetic electron transport (Genty *et al.* 1989).  $F_v/F_m$  in T2

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**Abbreviations:** Chl – chlorophyll;  $F_v/F_m$  – maximal photochemical efficiency of PS2 while all PS2 reaction centres are open;  $P_N$  – net photosynthetic rate; PS – photosystem;  $q_{NP}$  – non-photochemical quenching coefficient;  $q_P$  – photochemical quenching coefficient;  $\Phi_{PS2}$  – actual quantum yield of PS2 under actinic irradiation.

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was the greatest at 16 d (Fig. 1A). After 18 d,  $F_v/F_m$  in T2 kept on decreasing, but in other treatments it increased.  $F_v/F_m$  in T3 was the greatest and in T2 the smallest at 24 d. Thus  $F_v/F_m$  of tobacco could be increased by growing with both  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ .  $\text{NH}_4\text{-N}$  can enhance  $F_v/F_m$  in a short period, but as the growth of tobacco continues,  $F_v/F_m$  is reduced by the excessive  $\text{NH}_4\text{-N}$ .

$\Phi_{\text{PS}2}$  reflects the ratio of energy used for transporting photosynthetic electrons to photon energy absorbed by leaves. High  $\Phi_{\text{PS}2}$  indicates a high efficiency of photon energy transformation and more energy accumulated for the dark reaction (Schreiber *et al.* 1986). Both  $F_v/F_m$  and  $\Phi_{\text{PS}2}$  in T2 were the greatest at 16 d (Fig. 1A, B).  $F_v/F_m$  in T1 was the smallest, but its  $\Phi_{\text{PS}2}$  was greater than  $\Phi_{\text{PS}2}$  in T3. That shows that  $\text{NO}_3\text{-N}$  can control sufficient use of latent photochemical ability. After 18 d,  $\Phi_{\text{PS}2}$  in T2 decreased slowly, but in T1 and T3 it increased slowly.  $\Phi_{\text{PS}2}$  in T3 was the greatest at 24 d, and thus tobacco grown with the appropriate mixture of  $\text{NO}_3\text{-N}$  with  $\text{NH}_4\text{-N}$  can sufficiently utilize photon energy absorbed by leaves, and increase the efficiency of energy trans-

formation to provide enough reducing power for photosynthetic carbon assimilation.

$q_p$  is a measure of the oxidation condition of the original electron receiver  $Q_A$  in PS2, and represents the fraction of open PS2 reaction centres, so it can reflect the ratio of energy used by photochemical reactions to the energy absorbed by antenna pigments in PS2, and is related to carbon assimilation (Gilmore and Yamamoto 1991). High  $q_p$  is advantageous to the separation of electric charge in the reaction centre, and the ability to transport electrons and the quantum yield of PS2 are enhanced.  $q_p$  in T2 was the greatest in the three treatments (Fig. 1C). After 18 d,  $q_p$  in T2 decreased slowly, and  $q_p$  in T1 and T3 slowly increased. Thus  $\text{NH}_4\text{-N}$  can enhance the activity of electron transport in PS2 during short term. As the growth of tobacco continues, the activity of transporting electrons in T2 descends gradually, and that in T1 and T3 ascends slowly.

The  $q_{\text{NP}}$  is often used to estimate the ability of plants to safely dissipate excessive excitation energy (Härtel and Lokstein 1995). In other words, it can represent the

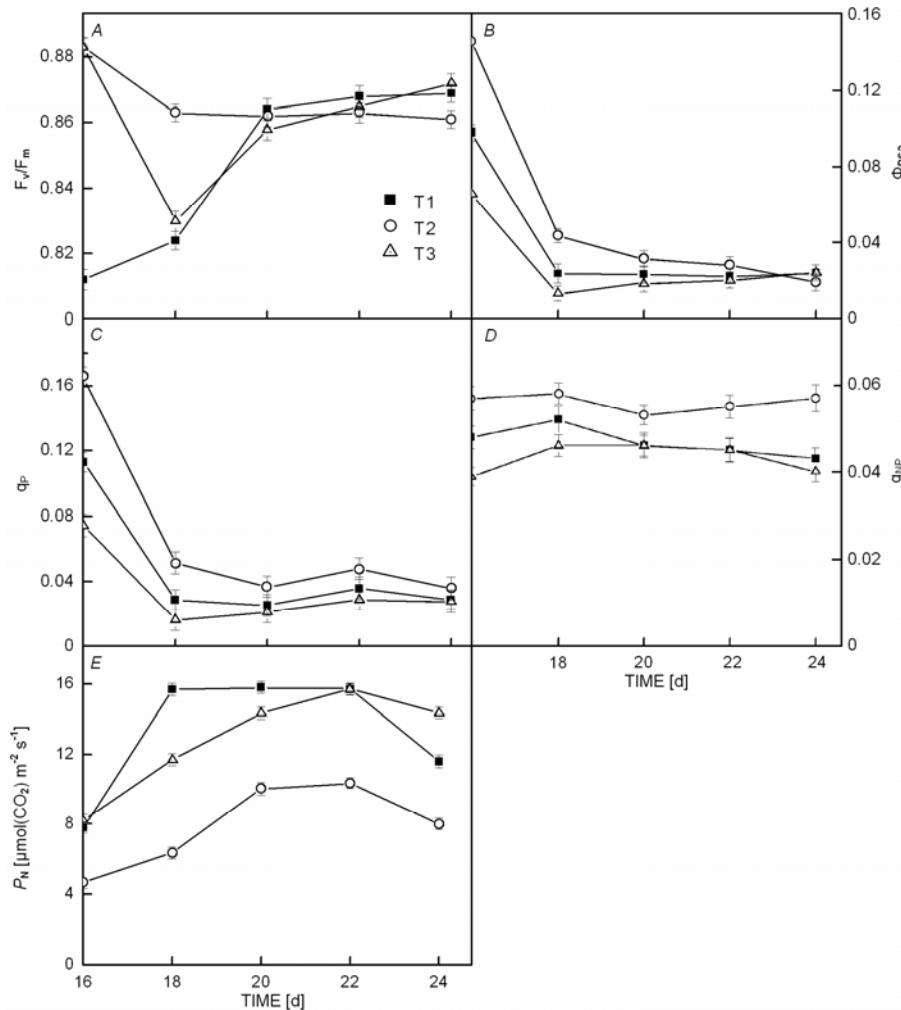


Fig. 1. The influence of different nitrogen forms (T1, T2, T3 – see the text) on  $F_v/F_m$  (A), actual quantum yield of photosystem 2,  $\Phi_{\text{PS}2}$  (B), fluorescence quenchings  $q_p$  (C) and  $q_{\text{NP}}$  (D), and net photosynthetic rate,  $P_N$  (E).

energy dissipated as heat energy which can not be utilized to transport photosynthetic electrons.  $q_{NP}$  in T2 was the greatest at all times (Fig. 1D), which indicated that  $NH_4^+$ -N can promote the thermal dissipation, so that tobacco does not utilize efficiently the photon energy absorbed by antenna pigment in PS2 for photosynthesis. The  $q_{NP}$  in T3 was the smallest, which indicated that the appropriate mixture of  $NO_3^-$ -N with  $NH_4^+$ -N can effectively reduce the thermal dissipation and utilize energy absorbed by antenna pigment in PS2 for photosynthesis.

$P_N$  was consistently the smallest when plants were grown only with  $NH_4^+$ -N (Fig. 1E).  $P_N$  in plants grown only with  $NO_3^-$ -N was greatest from 18 to 22 d after being transplanted, and when grown with both N forms it was greatest from 22 to 24 d after being transplanted. Thus this fertilization was advantageous for photosynthesis.

The different N forms have different influence on physiological and biochemical processes, and also have some influence on the metabolism of carbon and nitrogen. The different N forms affect the content and function of PS1 (photosystem 1) and PS2, and consequently influence the conversion of photochemical energy (Dong *et al.* 2002).  $F_v/F_m$  and  $\Phi_{PS2}$  in the treatment with only  $NH_4^+$ -N were greater at the early growth stage, and then all decreased showing a rapid promotion of the activity and photochemical reaction of PS2. The assimilation of N is an important process using the reducing power of the light reaction.  $NH_4^+$ -N absorbed by plant can be directly

used, but absorbed  $NO_3^-$ -N cannot be used until it is deoxidized to  $NH_4^+$ -N, and the process consumes energy and reducing power (Zhang *et al.* 1995). Therefore,  $NH_4^+$ -N can be utilized rapidly at the early stage, which is in favour of chloroplast synthesis and can promote photochemical efficiency. As the growth of tobacco continues, the excessive  $NH_4^+$ -N can be accumulated in plants so as to damage the membrane configuration and uncouple photophosphorylation with non-photophosphorylation. Thereby, the fixation of  $CO_2$  is reduced and photochemical efficiency decreases (Zhang *et al.* 1995). An appropriate amount of  $NO_3^-$ -N can improve photochemical efficiency of PS2.

The leaves of tobacco cultured with only  $NH_4^+$ -N were dark green, but their  $P_N$  was the smallest at all times. There are two reasons for this. The first is that the excessive  $NH_4^+$ -N can damage photosynthesis organs and decrease photochemical efficiency, the other is that the excessive  $NH_4^+$ -N can markedly increase the ability of chloroplasts to dissipate the excessive energy. So they can not efficiently utilize the photon energy absorbed by pigments for photosynthesis.  $NH_4^+$ -N partly replacing  $NO_3^-$ -N decreases the consumption of energy and reducing power, while  $NO_3^-$ -N partly replacing  $NH_4^+$ -N relieves metabolic disorder induced by the excessive  $NH_4^+$ -N and makes the physiological metabolism in tobacco balanceable.

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