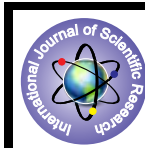


Photosynthesis of Flue-Cured Tobacco (*Nicotiana Tabacum* L.) Plants Subjected to Different Fertilization Rates in Huila, Colombia



Agronomy

KEYWORDS : macronutrients, nitrogen, plant production, photosystem II maximum efficiency.

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ABSTRACT

*A balanced management of nutrients in a crop involves several aspects, such as the application of the essential elements for crop growth, taking into account the soil conditions that influence their availability, time, sources and doses according to crop needs. The lack of any of these elements has both direct and indirect effects. The aim of this study was to evaluate physiological parameters, such as photosynthesis (Pn) and maximum efficiency of the photosystem II (Fv/Fm), depending on the nutritional status of tobacco (*Nicotiana tabacum* L.) plants in the municipalities of Campoalegre and Garzón in the province of Huila, Colombia. The photosynthesis was primarily affected at the initial stages of the crop. Net photosynthesis, and maximum PSII efficiency of tobacco were affected mainly by the location evaluated. The fertilizer application rate influenced the Fv/Fm, and the fertilization source influenced Pn, y Fv/Fm.*

INTRODUCTION

Competitive agricultural production of tobacco (*Nicotiana tabacum* L.) in the tropics requires the growers to be more efficient in the production, i.e. procure better production and quality at the lowest cost. Also, it requires proper fertilization management due to economic and environmental constraints (Rojo, 2006). In fertilization practices of flue-cured tobacco, potassium is the "key quality factor", whereas nitrogen is the "key yield factor" (Venegas, 1998). The potassium content in a tobacco leaf is highly correlated with the quality of tobacco leaf and cigarette safety (sugar content, nicotine and combustibility) (Gurumurthy & Vageesh, 2007; Yang et al., 2007), improves the taste and combustibility of the leaves, lowers the tar content of the smoke, and leads to good physical properties of the cured leaves, such as color, body, texture and elasticity (Zou et al., 2005). Nitrogen is the element which has the highest effect on growth and quality of flue-cured tobacco compared to other essential nutrients (Parker, 2009; Smith, 2009). Nitrogen is directly related to the vegetative development of the plant and specifically determines the development of the leaf, its aroma and flavor characteristics, and the taste of the smoke (Marchetti et al. 2006; Marambe & Sangakkara, 2008; Smith, 2009). Phosphorous is most essential element at the early stages of development of the tobacco plant, influencing the development of roots, plant growth, and the growth of first leaves as well as their quality. This element enhances the color of the tobacco leaf; it is related positively to the sugar content of the leaves, one of their quality parameters (Smith, 2009). Magnesium is an essential component in the production of chlorophyll, thus, it plays an important role in photosynthetic processes and plant development (Smith, 2009). For tobacco, increased magnesium content up to 2% improves leaf combustibility and appearance (color and texture) of the ash, resulting in a porous ash, loose and light colored, which improves combustion. Sulfur affects the combustibility, aroma and may decrease the rate of alkalinity of the ash (Rojo, 2006; Smith, 2009).

Photosynthesis is the main physiological process that runs the plant growth and crop productivity, and includes many other processes in plants. Nitrogen deficiency affects photosynthesis of plants in many crops. This is because there is a direct relationship between the photosynthetic capacity and the amount of N per unit leaf area, therefore a deficiency of this element restricts the translocation within the plant by a decrease in growth (Boussadia et al. 2010). Likewise, the proportion of photoas-

simulates aimed to developing storage substances, such as sugars and starch, decrease the synthesis of nitrogen compounds (Ballari, 2005). Leaf area and leaf photosynthesis rate are directly associated with the production of plant dry matter. Foliar N content and chlorophyll concentrations are important physiological parameters to detect the status of plant N culture (Zhao et al., 2005).

Given the theoretical importance of mineral nutrition for photosynthesis and plant production, it is important to develop appropriate strategies for the cultivation of tobacco under local conditions in Colombia. Therefore, the objective of this study was to evaluate physiological parameters such as photosynthesis and maximum photosynthetic efficiency, depending on the nutritional status of the plants.

MATERIALS AND METHODS

The study was conducted in 2010, in two tobacco farms located in the province of Huila (Colombia), in the municipalities of Campoalegre (525 m.a.s.l., average temperature of 28 °C, relative humidity of 68% and average solar radiation of 32 MJ m⁻²) and Garzón (790 m.a.s.l., average temperature of 25 °C, relative humidity of 74% and average solar radiation of 28 MJ m⁻²). These municipalities exhibited sandy loam soils with slight and moderate acidic pH, respectively, P, K, Mg, S, and Cl contents challenging for cultivation, and ideal texture, pH and N percentage. The material used was tobacco (*Nicotiana tabacum* L.) Virginia variety NC297, the seedbed was prepared and when the plants had 5 true leaves they were transplanted with a planting density of 20,833 plants ha⁻¹, distributed in rows 1.2 m wide and 0.4 m between plants, each experimental unit area of 57.6 m². Crop management was performed in accordance with the production practices of each study area.

The trials were established under a completely randomized design with a 3x2 factorial arrangement. Seven treatments in three replicates were used; they corresponded to six fertilization alternatives and one conventional treatment. The conventional treatment corresponded to the current fertilization recommended by the company Protobaco (Plaza et al., 2011) and used by the growers. The alternatives corresponded to combining three fertilization doses and two sources (Table 1), maintaining a proportion of 50:50 NO₃⁻: NH₄⁺ for the N forms. The data of soil analysis according the methodology of ICA (1992) and Gómez (2005) were considered for selecting the fertilization doses that cor-

respond to 100%, 50%, or 150% of the recommended dose. To make a recommendation on the amount of fertilizer, the levels of extraction of the mineral elements by tobacco were applied as reported by Ballari (2005) with an expected production of 3,200 kg ha⁻¹. Fertilizer applications were performed according to the absorption curves of mineral elements by the plants (Rojo, 2006), fractionated in two applications for the two municipalities at 8 DAT (days after transplantation) and 35 DAT.

Table 1. Treatments for each study area, in terms of amount of product applied (kg ha⁻¹).

PRODUCTS (kg ha ⁻¹)								
Trat	ID*	Dosis	17-9-18-3	13:3:43	21:11:7,5	AMS	Sulfato K	DAP
CAMPOALEGRE								
T1	a50	50	275	288	175	50		
T2	a100	100	550	575	350	100		
T3	a150	150	825	863	525	150		
T4	b50	50	550	163	75			
T5	b100	100	1100	325	150			
T6	b150	150	1650	488	225			
T7	Control	Ctrl	750	50		50	100	50
GARZÓN								
T1	a50	50	250	288	63	25		
T2	a100	100	500	575	125	50		
T3	a150	150	750	863	188	75		
T4	b50	50	475	125	150			
T5	b100	100	950	250	300			
T6	b150	150	1425	375	450			
T7	Control	Ctrl	790	105		155	155	

Abbreviations: *a: Use of AMS, b: Without the use of AMS. 17-9-18-3 contains N:P₂O₅:K₂O:MgO:S (17:9:18:3:6) (relation N-NH₄:N-NO₃ = 60:40). 13:3:43 contains N:P₂O₅:K₂O (relation N-NH₄:N-NO₃ = 5:95). 21:11:7.5 contains N:CaO:MgO (relation N-NH₄:N-NO₃ = 50:50). Ammonium sulphate (AMS) contains N:S in the proportion of 21:24 (relation N-NH₄:N-NO₃ = 100:0). Potassium sulphate contains K₂O:S in the proportion of 50:17. DAP contains N:P₂O₅ in the proportion of 18:46 (relation N-NH₄:N-NO₃ = 100:0).

Physiological parameters

During crop development three measurements of physiological parameters that indicate the nutritional status of the plants were conducted. These measurements were performed at different stages of crop development, the first during slow growth phase (24 DAT) BBCH code 1105, the second during rapid growth stage (44 DAT) BBCH code 1110, and third during flowering (74 DAT) BBCH code 59 (Coresta, 2009). For the physiological analysis, the 5th leaf counted from the apical meristem was taken, which was fully expanded. The gas exchange measurements (P_n) were performed using the portable photosynthesis system LI-6200 (LI-COR Inc., Lincoln, NE, USA) in each sample three replicates were made which corresponded to three times in the day: morning (9:00 to 10:00 h), noon (12:00 to 13:00 h) and afternoon (15:00 to 16:00 h). The maximum efficiency of the photosystem II (Fv/Fm) was measured using a continuous excitation chlorophyll fluorescence analyzer (Handy PEA, Hansatech Instruments, Kings Lynn, UK). The leaves previously adapted to the dark for 15 min, then exposed to a maximum light intensity

of 3.000 m² s⁻¹ mol. The maximum photosynthetic efficiency was measured in the morning between 9:00 and 10:00 h.

Statistical analysis

An analysis of variance (ANOVA) was conducted with SAS * statistical software v. 8.1e (SAS Institute Inc., Cary, NC) and when significance was found, an LSD mean comparison test was conducted with a reliability of 95%.

RESULTS

Photosynthesis

In the slow growth stage, there were highly significant differences found between the interactions of municipality/time of the day and municipality/ fertilizer source (P < 0.05). In general, the municipality with the highest values of photosynthesis was Campoalegre with values greater than 3 mol CO₂ m⁻² s⁻¹ and for this municipality it was found the highest photosynthetic rate (8.25 mol CO₂ m⁻² s⁻¹) (Figure 1a). Furthermore, the use of ammonium sulphate in Campoalegre resulted in high values of 7 mol CO₂ m⁻² s⁻¹ (Figure 1b).

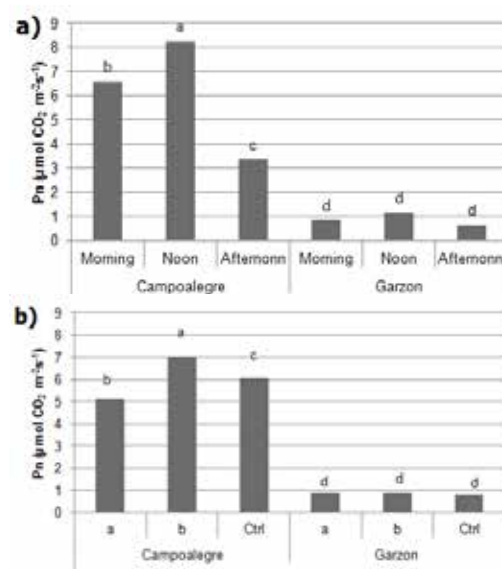


Figure 1. Net photosynthetic rate P_n (μmol CO₂ m⁻² s⁻¹) in flue-cured tobacco leaves in slow growth stage. a). Municipality/time of the day interaction. b). Municipality-fertilizer source interaction (a: use of SAM, b: not using SAM, Ctrl: control). Distinct letters in the column indicate significant differences according to LSD's test (P ≤ 0.05).

In the rapid growth stage, there were significant differences observed in the municipality/fertilization interaction (P < 0.01), where the town of Garzón had photosynthetic rate values higher than 12 mol CO₂ m⁻² s⁻¹ and the highest value of P_n for this municipality was at 100% dose fertilization reaching 14 mol CO₂ m⁻² s⁻¹ (Figure 2).

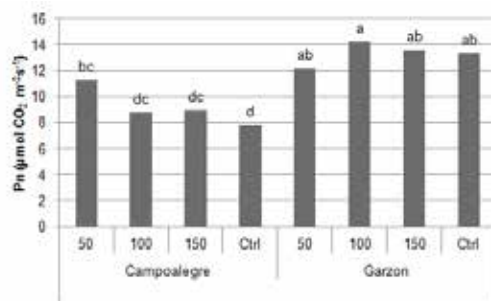


Figure 2. Net photosynthetic rate P_n (μmol CO₂ m⁻² s⁻¹) in flue-cured tobacco leaves in rapid growth stage.

flue-cured tobacco leaves in rapid growth stage. Municipality/dose interaction. Distinct letters in the column indicate significant differences according to LSD's test ($P \leq 0.05$).

For rapid growth and flowering, there were statistical differences observed between the hours assessed ($P < 0.01$) and the morning hours were the time with the highest net photosynthesis for growing tobacco ($13 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $21 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively) (Figure 3a and b).

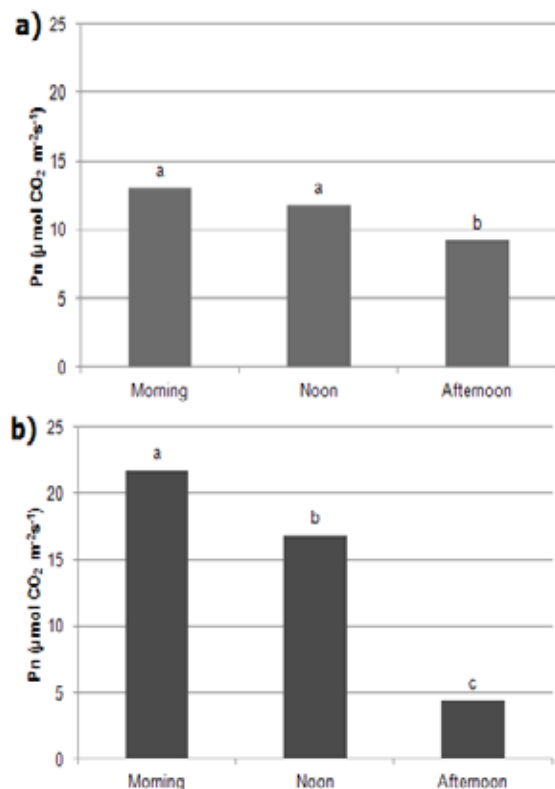


Figure 3. Net photosynthetic rate P_n ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) flue-cured tobacco leaves at different times of day. a). Rapid growth stage (44 DAT). b). Flowering stage (74 DAT). Distinct letters in the column indicate significant differences according to LSD's test ($P \leq 0.05$).

Maximum efficiency of photosystem II

In the slow growth stage (24 DAT), there were highly significant differences detected between the two municipalities evaluated ($P < 0.01$), in the municipality/fertilization interaction ($P < 0.01$) and significant differences in municipality/fertilizer source/dose interaction ($P < 0.05$). Garzón municipality had the highest values of Fv/Fm for all treatments. The treatments with the highest values of maximum efficiency of PSII were achieved using SAM at 150% (a150) in the town of Garzón and the non-application of AMS to 100% of the dose (b100) in the municipality of Campoalegre (0.79 and 0.78, respectively) (Table 2). In the rapid growth stage (44 DAT), there were only significant differences found between the municipalities of study ($P < 0.01$), being the town of Garzón the one that presented the highest values of Fv/Fm, 0.75 and above. In the flowering stage (76 DAT), these characters were not significantly different (Table 2).

Table 2. Effect of different sources and rates of fertilization evaluated on the maximum efficiency of photosystem II (Fv / Fm) in the two municipalities during crop development

Source	Dose	Slow growth		Rapid growth		Flowering	
		Campoalegre	Garzón	Campoalegre	Garzón	Campoalegre	Garzón
a	50	0.77 ab	0.78 ab	0.67	0.78	0.74	0.75
	100	0.74 b	0.76 ab	0.71	0.79	0.79	0.75
	150	0.69 c	0.79 a	0.66	0.80	0.80	0.78
b	50	0.68 c	0.76 ab	0.67	0.78	0.75	0.76
	100	0.79 a	0.77 ab	0.64	0.76	0.80	0.73
	150	0.69 c	0.77 ab	0.72	0.77	0.81	0.71
Control	Ctrl	0.69 c	0.77 ab	0.65	0.80	0.80	0.75
Source		NS		NS		NS	
Dose		NS		NS		NS	
Source-Dose		NS		NS		NS	
Municipality		***		***		NS	
Municipality-Source		NS		NS		NS	
Municipality-Dose		***		NS		NS	
Municipality-Source-Dose		**		NS		NS	

a: Use of SAM, b: not using SAM. Distinct letters in the row indicate significant differences according to LSD's test ($P \leq 0.05$).

NS, Not significant ($P > 0.05$)

*Significance to $P < 0.05$

**Significance to $P < 0.01$

***Significance to $P < 0.001$

DISCUSSION

Net photosynthesis in tobacco was affected mainly by municipality evaluated, and Campoalegre presented the highest values of these variables. This is because environmental conditions in each area differed in temperature, solar radiation and relative humidity. The municipality of Campoalegre has more solar radiation, higher average temperature and lower relative humidity, over that of Garzón. The growth and development of plants depends on various environmental variables, such as temperature, relative humidity, light intensity, and availability of water and essential minerals (Azcón-Bieto et al., 2004). These environmental factors also modify the photosynthetic capacity of plants significantly. An increased photosynthetic production occurs with higher radiation levels (Niinemets, 2007), temperature increments also increase photosynthetic rates to the point of denaturation of enzymes and the destruction of the photosystems (Evans, 1989). Similarly, the availability of nutrients in the soil and light conditions affect the morpho-physiology, the distribution of biomass (Hernández et al., 2009), and also photosynthetic and respiratory processes of plants (Azcón-Bieto et al., 2004).

Net photosynthesis reached the highest value ($14 \text{ CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at the recommended dose (100%) aside from the fertilizer source,

at the rapid growth stage. Similar results were obtained in *Pinus palustris*, where P_n was significantly higher with high nutrient availability with respect to low fertilization levels (Jose et al., 2003). Photosynthetic efficiency increases when augmenting N dose, may be related to the ability of the plant to optimize photosynthetic capacity by boosting nitrogen supplement (Sanclemente & Peña, 2008). However, a high availability of elements in the soil does not translate directly into higher plant photosynthesis (Jose et al., 2003). In olive, a decrease in the proportion of nitrogen applied affect the photosynthetic capacity of the plant, reducing between 16% and 28% CO_2 assimilation with 60% less of this element. The decrease in photosynthetic capacity is not only associated with the direct effects of N deficiency but it also serves as a negative feedback mechanism of carbohydrates from the leaf (Boussadia et al., 2010).

A decrease in N in fertilization programs diminishes growth and photosynthesis, because it reduces leaf production, and, consequently, individual and total leaf area; this results in a reduction of the area that intercepts light for photosynthesis (Azcón-Bieto et al., 2004). A decrease in photosynthesis rate under conditions of nitrogen deficiency is attributed to a reduction in chlorophyll content and Rubisco activity because 75% of the N foliar is in chloroplasts and most of this is in the ribulose biphosphate carboxylase (Tóth et al., 2002). However, under high nitrogen levels conditions there could be also decreased photosynthetic activity in plants, indicating that the absorption rate per unit of chlorophyll is inhibited with an increase in supply of the element (Tóth et al., 2002). There is a close relationship between the nitrogen form applied (nitrate and/or ammonia) and the rate of photosynthesis, the growth, yield and quality of the tobacco plant (Guo et al., 2006). Studies carried out on tobacco by Geiger et al. (1999) show that plants fertilized with NH_4^+ have higher CO_2 assimilation rate and stomatal conductance than those fertilized with NO_3^- . However, Guo et al. (2006) showed that when the two application forms of nitrogen are used, the rate of photosynthesis and the highest photosynthetic efficiency were not affected and can be improved.

Photosynthetic efficiency may be limited by the decrease in the content of Rubisco and leaf chlorophyll as well as the quantum yield of photosynthesis. The correlation between the nitrogen content and photosynthetic efficiency varies depending on the habitat of the plant and environmental factors, such as temperature and radiation (Tóth et al., 2002). A raise in photosynthetic efficiency with increasing doses of N can be associated with the ability of the plant to optimize the photosynthetic capacity through an improved nitrogen supply (Sanclemente & Peña, 2008). A reduction in photosynthetic rate can result in a significant reduction in the growth of tobacco plants (Karkanis et al., 2007) and, therefore, in performance. Pieters et al. (2001) reports a decrease in photosynthetic rate when there is a deficiency of phosphorus. The main effect of potassium deficiency in cotton plants is the reduction of photosynthesis and leaf area development, consequently resulting in a decrease in biomass production and changes in the distribution of biomass among plant organs (Reddy & Zhao, 2005).

Various environmental stress factors such as high temperatures, drought, changes in light intensity, nutritional deficiencies, the presence of heavy metals, and herbicides among others, affect directly or indirectly the function of photosystem II, which modifies the emission fluorescence, these changes are used to quantify responses to the presence of stress (Gonzalez et al., 2008). In this study, the municipality of Garzón had the highest photosystem II (Fv/Fm) maximum efficiency values, higher than 0.75. In the slow growth stage, the treatment with higher efficiency of PSII SAM was obtained using 150% of the dose (0.79) in Garzón. These results are consistent with research conducted in corn, where greater nitrogen dosages obtained a higher value of fluo-

rescence chlorophyll, stomatal conductance, and transpiration rate (Tóth et al., 2002; Karkanis et al., 2007). In rice, an efficient supply of N shows a higher response rate of photosynthesis and Fv/Fm versus inefficient N (Wei et al., 2009). Furthermore, in wheat, there were no differences in the maximum efficiency of photosystem II when applications with high or low in N (Cai et al., 2008), although the P_n increased with higher N applications compared to low applications (Shangguan et al. 2000; Cai et al., 2008). The physiological responses of the plant suggest that the photochemical activity of the Photosystem II does not depend only on the availability of nitrogen; a deficiency in this element, however, directly affects the content of chlorophyll and, therefore, the effective growth rate of the plant (Sanclemente & Peña, 2008).

CONCLUSIONS

There is several growth and development alterations in tobacco cultivation, which are directly related with yield and commercial quality of the leaves, said alterations have been associated to macro- and micronutrient deficiencies. The results obtained allow concluding that the physiological variables of the flue-cured tobacco plants were affected by the treatments evaluated, mainly in the early stages of crop development. Net photosynthesis is primarily affected by the municipality evaluated, where Campalegre had the highest values for these variables. At the rapid growth stage, net photosynthesis reached the highest value at the recommended dose independently of the fertilizer source. The most photosynthetic efficient treatment was using AMS at 150% dose in the municipality of Garzón.

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