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Effects of Transplanting Time on 15-Nitrogen Utilization and Industrial Quality of Flue-Cured Tobacco

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Abstract: Nicotine concentration is a key index and directly affects the industrial quality and availability of flue-cured tobacco (FCT). Seedlings transplanted at different times were subjected to different climatic conditions, which were closely correlated with the growth, development, and nicotine synthesis of FCT. An appropriate transplanting time is imperative for ensuring high-quality tobaccos. Hence, a ^{15}N tracing experiment in the field (which included three treatments: FCT seedlings transplanted on 5th, 15th, and 25th May) was carried out to evaluate the influence of different transplanting dates on ^{15}N utilization and nicotine concentration in FCT leaves. Results showed that compared to the tobacco seedlings transplanted on 5th May, the seedlings transplanted on 15th and 25th May increased the dry matter weight by 9.86 - 87.5 % and N uptake by 24.4 - 36.9 % in shoots during the field growing periods of FCT plants. However, it was notable that postponing the transplanting time of FCT seedlings decreased the proportion of nicotine-N to total N (26.9 - 50.6 %), ^{15}N abundance in total N (7.27 - 40.7 %), and nicotine-^{15}N abundance in total nicotine-N (7.30 - 35.7 %), thus inducing a noticeable reduction of 24.3 - 35.8 % in the nicotine concentration in FCT leaves. Hence, delaying transplanting time promoted dry matter and N accumulation, while it significantly decreased the nicotine concentration in FCT leaves, which is of great importance in improving the industrial quality and availability of tobaccos.

Keywords: cash crop, agronomy measurement, nicotine, nutrient use efficiency, isotope.

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INTRODUCTION

Flue-cured tobacco (FCT) is N-sensitive and has a stringent requirement for N. In tobacco, N uptake is low during the first three weeks after transplanting, and then sharply increases between the third and eighth weeks. About 80 % of total N is taken up by the first eight weeks (Collins and Hawks, 1993). However, lower N uptake after topping is beneficial to maintain low nicotine concentration in FCT leaves, since nicotine accumulation in leaves mainly occurs during late stages of growth, especially during the period after removing the apex (Mumba and Banda, 1990).

Nicotine, a unique alkaloid of tobaccos, is the secondary metabolite that helps in adaptation to biotic stress and accounts for about 90 % of the total alkaloid content and 0.60 to 3.00 % of dry matter of tobaccos (Baldwin, 1988; Doolittle et al., 1995; Hoffmann and Hoffmann, 1998). Nicotine mainly synthesized in roots and accumulated in leaves (Yoshida and Takahashi, 1961). Nicotine concentration is one of the most important indexes of leaf quality and directly affects its industrial availability and the safety of FCT. According to Nagarajan and Prasadrao (2004), the nicotine concentration in tobacco leaves should be limited to 1.75 - 2.00 %. Many factors (e.g., agronomic traits, climate conditions, etc.) influence the nicotine concentration of tobaccos (Tso, 1969).

Nitrogen is closely correlated with nicotine concentration (Karaivazoglou et al., 2007; Ju et al., 2008) since N is 17.3 % of the molecular weight of nicotine (Collins and Hawks, 1993). Lack of available N in the soil decreases the amount of jasmonic acid (JA), which is an important signaling substance for regulating nicotine synthesis in tobacco roots, and further decreases the nicotine concentration in tobacco leaves (Lou and Baldwin, 2004). Excessive available soil N increases nicotine concentration in tobacco leaves after topping (Xi et al., 2005; Bilalis et al., 2009). The application time of N fertilizer also affects the quality of tobacco leaves (Ahmed et al., 1986). Compared with one-time fertilization, split application of N fertilizer improved N accumulation in tobaccos (Elvira et al., 2004) and thus greatly increased the nicotine concentration in the upper leaf of FCT (Zuo, 1993).

Climatic conditions also play important roles in nicotine concentration of the FCT leaf. Under the condition of relatively low soil N content, additional water decreases the nicotine concentration, due to the dilution effect. However, if soil N content is relatively high, additional water increases nicotine concentration as the N uptake under these circumstances exceeds the dilution effect (Biglouei et al., 2010). Moreover, a prolonged photoperiod or short-wavelength sunlight significantly increased nicotine concentration (Tso et al., 1970; Kartusch and Mittendorfer, 1990), but very strong or very weak light intensity led to the highest nicotine concentration in tobacco leaf (Tso, 1990).

An appropriate transplanting time is conducive to taking advantage of the best climatic conditions and is imperative for producing sound tobaccos (Patel et al., 1989). We hypothesized that flue-cured tobacco plants transplanted on different dates will subject to different climatic conditions (e.g., sunlight, temperature, rainfall, etc.) and in turn influenced the leaf qualities during the field growth period as well as its industrial availability. Hence, the aim of this study was to understand the influence of different transplanting time on ^{15}N utilization and nicotine content in FCT plants; and to evaluate the contributions of fertilizer-^{15}N and soil-N to nicotine synthesis.

MATERIALS AND METHODS

Experimental site and soil

A field experiment was carried out at Xiangyang, China (31° 28’ N, 111° 15' E, 903 m a.s.l.). The experimental site has a subtropical monsoon climate, characterized by heavy rain from May to August and seasonal drought from October to December. The duration of average annual solar radiation is 1,875.6 h; the mean daily temperature is 17.6 - 25.5 °C;

the frost-free period is 214.6 d; and the average annual accumulated temperature over 10 °C is 3,840.6 °C. The average daily temperature and rainfall at the experimental site from April to September are shown in figure 1. The experimental soil is classified as Udalf (Soil Survey Staff, 2014).

Soil samples were air-dried at room temperature for two weeks and passed through a 2 mm sieve prior to laboratory analysis. Soil pH (soil:water ratio was 1:2.5), total N (Kjeldahl method), extractable P (the Olsen method), and available K (with ammonium acetate) were determined following procedures described by Page et al. (1982). The organic C content was determined according to the method described by Walkley and Black (1934). Soil particle fractions of different sizes were obtained via low-energy sonication and a combination of wet sieving and centrifugation, as described by Stemmer et al. (1998) and Sessitsch et al. (2001), with minor modifications. In brief, fresh soil was dispersed in distilled water to allow the particle-size fractions (PSFs) to become saturated. The soil-water suspension was then dispersed via low-energy sonication (output energy of 170 J g^{-1} dry soil). The coarse sand fraction (2,000-200 µm) was separated via wet sieving. The 20-200 µm fraction was subsequently obtained through siphonage and sedimentation and was considered the fine sand fraction. The remainder of the sample was centrifuged to collect the 2-20 µm fraction (silt), and the supernatant was centrifuged again to collect the <2 µm fraction (clay). The initial physicochemical properties of the topsoil (0.00-0.20 m) were shown in table 1.

Plant materials and treatments

Flue-cured tobacco (cv. K326) seeds were germinated in a mixture which was consisted of 50 % (w/w) carbonized rice husk, 25 % (w/w) silver sand, and 25 % (w/w) perlite in foam salver, and then cultured in a naturally lighted plastic-covered greenhouse for 75 days.

The field experiment included a macroplot experiment and a microplot experiment. The macroplot experiment was designed as a randomized complete block with three

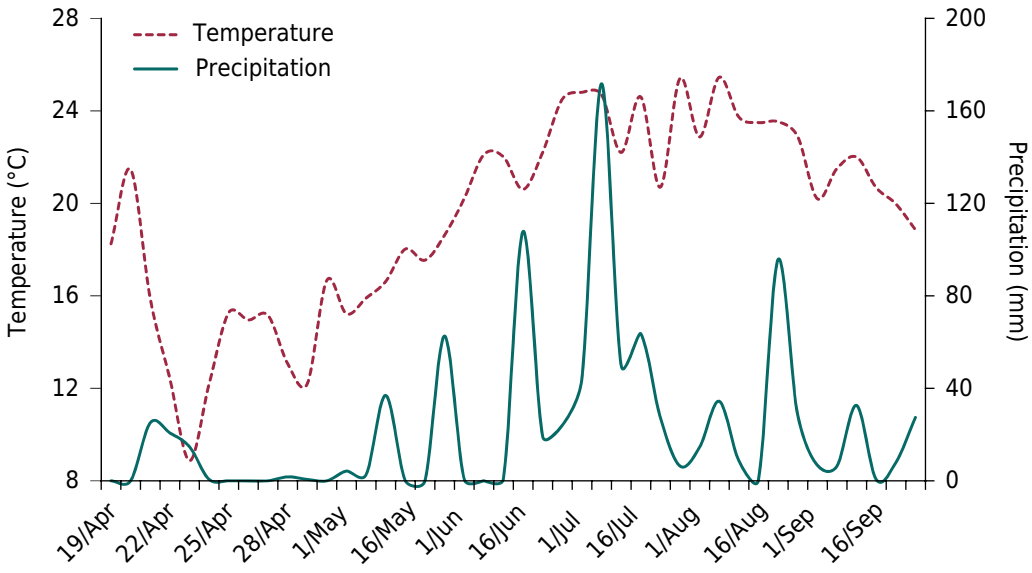

Figure 1. Temperature and precipitation from April to September at the experimental site.

Table 1. The initial physicochemical properties of the soil

pH (H_2O)	Soil organic matter	Total N	Alkali-hydrolysable N	P	K	Silt	Clay	Sand
	g kg^{-1}		mg kg^{-1}			%		
6.21	52.6	1.68	110.2	12.9	119.0	22.3	65.3	12.4

Soil pH (soil:water ratio was 1:2.5). Total N (Kjeldahl method). Extractable P (Olsen method). Available K (Page et al., 1982). Soil organic matter (Walkley and Black, 1934). Soil particle fractions of different sizes were obtained via low-energy sonication and a combination of wet sieving and centrifugation, as described by Stemmer et al. (1998) and Sessitsch et al. (2001).

replications. The experimental design included three different transplanting dates for FCT seedlings: May 5th, 15th, and 25th. Each plot was 42.9 m^2 and had 60 plants. Plots consisted of four rows, with a 1.30 m row spacing and 0.55 m plant spacing, i.e., 13,986 plants per ha. Seedlings were planted and divided into a harvesting area (40 plants) and sampling area (20 plants). The fertilizer application rate to FCT plants was 72 kg ha^{-1} N (NH_4NO_3, 35 % N), 86.4 kg ha^{-1} P (super phosphate, 12 % P_2O_5), and 216 kg ha^{-1} K (K_2SO_4, 50 % K_2O). All the P fertilizer and 70 % of the N and K fertilizers were applied as basal fertilizer to topsoil 15 days before transplanting FCTs. The remaining 30 % of N and K fertilizers were applied as topdressing 15 days after transplanting.

The microplots (consisting of one FCT plant each) were set within the macroplots. Part of an FCT row was enclosed with polyvinyl chloride (PVC) panels (0.70 m long and 0.40 m high), which were inserted into the macroplot with the top edge at 0.15 m above the ground, separating it from its neighboring plot. Three FCT plants (i.e., three microplots) in each treatment received application of ^{15}N-labeled fertilizer at a rate of 3.61 g ^{15}N-NH_4NO_3, 6.18 g P, and 10.8 g K per plant as basal fertilization after dilution in 100 mL of water; and 1.54 g ^{14}N-NH_4NO_3 plus 4.62 g K per plant were used as topdressing. The ^{15}N was provided as $^{15}NH_4{}^{15}NO_3$ (10.28 atom % excess), produced in the Research Institute of the Chemical Industry in Shanghai, China.

Sampling and determination

One FCT plant was taken at the rosette, topping, and maturity stage in the macroplot and in the microplot. The plant was divided into four parts, namely, the upper, middle, and lower leaf and the stem (the plant at the rosette stage was taken as one sample). All plant parts were de-enzymed at 105 °C for 30 min, dried at 70 °C, weighed, and pulverized, and then finely ground and passed through a 0.25 mm sieve prior to laboratory analysis.

To determine total N content, a sample of 0.3 g was digested, distilled, and titrated according to the semi-micro-Kjeldahl method (Lu, 2000). To determine ^{15}N abundance, 0.5-1.0 g samples were used. After titration, the solution was condensed to 1-3 mL in a water bath at 100 °C. The ^{15}N abundance was determined using the method of Buresh et al. (1982), by Isotope Ratio Mass Spectrometry (F2.32 innigan-Mat-251, Mass Spectrometers, Finnigan, Germany).

Nicotine concentration was determined through steam distillation and ultraviolet spectrophotometry (Al-Tamrah, 1999). Briefly, 0.5 g of dry sample was weighed in a clean, dry glass tube of 5 cm inner diameter, to which 20 mL distilled water and 10 mL NaOH 30 % (w/v) were added. The tube was placed in a distillation device and a 250 mL flat-bottomed flask was used to collect the distilled nicotine solution. Distilled water was added to complete the solution up to 250 mL, and it was then analyzed colorimetrically at 236, 259, and 282 nm respectively using a spectrophotometer (Shimadzu UV-2201, Japan). The nicotine concentration was expressed as a percentage of the tissue dry weight (%).

To measure the ^{15}N abundance in nicotine-N, 1-5 g of the dry samples were weighed, and the nicotine distillate was obtained as mentioned above. The distillate was concentrated into about 10 mL in a water bath at 100 °C, and the total nicotine-N and the amount of ^{15}N in nicotine-N was analyzed by the same method described above for determining total N content and ^{15}N abundance.

Data analysis

The proportion of total ^{15}N abundance to total N (*Ndf*) in FCT shoots was calculated using equation 1:

$$Ndf\ (\%) = \frac{a - b}{c - b} \times 100 \qquad \text{Eq. 1}$$

in which *a* is the atom% ^{15}N abundance in plant samples, *b* is the natural atom% ^{15}N abundance (0.365 atom %), and *c* is the atom% ^{15}N abundance of N fertilizer.

The ^{15}N accumulation in FCT shoots was calculated according to equation 2:

$$^{15}N(kg/ha) = W \times N\% \times Ndf\% \quad \text{Eq. 2}$$

in which W is the dry weight of the plant (kg ha^{-1}), N% is the N content of the plant samples, and Ndf% is the total ^{15}N abundance in total N.

The ^{15}N use efficiency (^{15}NUE) of the plant was calculated using equation 3:

$$^{15}NUE\ (\%) = \frac{^{15}N(kg/ha)}{R(kg/ha)} \times 100\ \% \quad \text{Eq. 3}$$

in which $^{15}N(kg/ha)$ is the total amount of fertilizer-^{15}N in the FCT shoots and R (kg ha^{-1}) is the amount of N fertilizer applied in each plot (kg ha^{-1} N).

The proportion of nicotine-N to total N (P_{nico}) was calculated according to equation 4:

$$P_{nico}\ (\%) = \frac{C_1 \times W \times 17.3\ \%}{C_2 \times W} \times 100\ \% \quad \text{Eq. 4}$$

in which C_1 is the nicotine concentration in the plant sample, W is the dry matter weight of the plant (kg ha^{-1}), C_2 is the N content in the plant sample, and 17.3 % is the N content in the molecular weight of nicotine.

The proportion of nicotine-^{15}N to total nicotine-N (P_{nicof}) was calculated according to equation 5:

$$P_{nicof}\ (\%) = \frac{A_{nico} - b}{c - b} \times 100\ \% \quad \text{Eq. 5}$$

in which A_{nico} is the atom% nicotine-^{15}N abundance in the plant sample, b is the natural atom % ^{15}N abundance (0.365 atom %), and c is the atom% ^{15}N abundance of N fertilizer.

Statistical analysis

Dry matter weight, N accumulation, ^{15}N abundance in total N, nicotine concentration, ^{15}N abundance in total nicotine-N, and ^{15}N use efficiency were obtained from three replicates of each treatment at different growing stages of the FCT plants. Values obtained from different treatments were subjected to ANOVA tests. Separation of means was performed on significant ANOVA tests by LSD ($p \leq 0.05$) using the SAS (version 9.1) package (SAS, 2004).

RESULTS

Dry matter and N accumulation, distribution in FCT shoots

It is evident that the dry matter weight (DWM) in the upper leaf and stem accounted for ≈ 30 % of total DWM, and ≈ 10 % of total DWM was distributed in the lower leaf (Table 2). Similarly, N accumulation in the upper leaf accounted for ≈ 35 % of total N in the FCT shoots; ≈ 30 % of total N was distributed in the stem; and less than 10 % of total N was distributed in the lower leaf. Both the DWM and N accumulation in the middle leaf accounted for ≈ 25 % of the total DWM and N amount in the shoots of FCT plants.

Postponing the transplanting time significantly increased the DWM and N accumulation at different growing stages of the FCT plants (Table 2). For example, compared to the transplanting date on May 5, postponing the transplanting date to May 25 significantly

increased the DWM by 87.5, 9.86, and 17.7 %, and the N accumulation by 36.9, 28.6, and 24.4 % at the rosette, topping, and maturity stages, respectively.

The proportion of total ^{15}N to total N in FCT shoots

The ^{15}N abundance in the total N of FCT shoots was 76.9 - 79.3 % at the rosette stage, but it was only 30.1 - 37.2 % at the maturity stage, which was a mean reduction of 51.6 - 62.0 %. At the maturity stage, the ^{15}N abundance in total N was 44.5 - 49.5 % in the lower leaf, but it was 22.6 - 38.1 % in the upper leaf, which declined by 23.8 - 50.8 % on average (Table 3).

Table 2. Effects of different transplanting time on dry matter weight and N accumulation of FCT shoots at different growing stages

Treat[(1)]	Rosette	Topping					Maturity				
	Shoot	UL	ML	LL	Stem	Shoot	UL	ML	LL	Stem	Shoot
					Dry matter weight (kg ha^{-1})						
5th	261.5± 24.4b	557.8± 88.9 b	691.7± 54.9b	430.2± 52.8b	847.4± 73.0b	2,527.0± 175.3b	1,072.8± 136.7b	957.2± 77.9b	397.6± 37.3b	1,097.4± 351.8c	3,525.0± 135.7c
15th	-	633.8± 33.0 a	715.2± 88.1ab	493.1± 31.0a	897.9± 60.3a	2,740.0± 52.1a	1,093.8± 46.7b	1,002.3± 25.6ab	512.3± 39.6a	1,134.3± 46.3b	3,742.7± 156.1b
25th	490.4± 19.7a	608.5± 50.9 a	797.4± 50.4a	483.3± 38.3a	886.9± 26.1ab	2,776.1± 61.9a	1,264.7± 189.5a	1,058.0± 40.5a	499.6± 44.1a	1,326.0± 80.4a	4,148.3± 653.0a
					N accumulation (kg ha^{-1})						
5th	13.0± 1.01b	12.3± 2.94b	9.65± 1.12b	4.48± 0.70b	9.23± 1.12a	35.7± 3.64b	22.1± 2.52b	12.0± 0.98b	3.92± 0.84b	17.8± 1.54c	55.8± 2.80b
15th	-	14.0± 1.96a	14.8± 0.98 a	6.15± 0.14a	9.79± 2.38a	44.8± 1.68a	24.3± 3.78a	16.4± 3.50a	6.01± 1.12a	19.3± 1.21b	65.9± 1.96a
25th	17.8± 0.32a	14.5± 1.68a	15.9± 0.70a	5.17± 0.28ab	10.2± 0.29a	45.9± 1.54a	24.9± 3.64a	17.2± 1.68a	4.76± 0.42ab	22.7± 0.98a	69.4± 3.64a

[(1)] Treatment. LL: lower leaf; ML: middle leaf; and UL: upper leaf. Values are means ± standard deviation (n =3). Means followed by different letters in the same column indicate significant difference at the 0.05 level. - : not tested.

Table 3. Effects of different transplanting time on total ^{15}N abundance in total N of FCT shoots at different growing stages (%)

Organs	May 5th	May 15th	May 25th
		total ^{15}N	
		%	
		Rosette stage	
Shoot	76.9±1.04a	-	79.3±2.61a
		Topping stage	
Upper leaf	49.6±2.24a	45.1±4.36b	38.8±3.71c
Middle leaf	59.5±2.37a	50.8±2.64b	43.3±1.70c
Lower leaf	67.8±2.03a	54.7±0.88b	55.1±4.13b
Stem	45.2±1.34a	34.7±4.28b	39.2±2.67b
Shoot	51.7±2.15a	47.3±1.89b	42.4±2.77c
		Maturity stage	
Upper leaf	38.1±2.05a	29.0±3.18b	22.6±1.71c
Middle leaf	47.1±9.45a	39.8±4.22b	31.7±0.25c
Lower leaf	49.5±1.64a	44.5±1.89b	45.9±1.57b
Stem	35.0±1.97a	29.8±4.90b	28.2±3.12b
Shoot	37.2±1.33a	31.6±0.98b	30.1±1.10b

Values are means ± standard deviation (n =3). Means followed by different letters in the same row indicate significant difference at the 0.05 level. -: not tested.

Delaying the transplanting time decreased the total ^{15}N abundances in total N at the topping and maturity stage of the FCT plant (Table 3). For instance, compared with the FCT seedlings transplanted on May 5th, delaying the transplanting time to May 15th and 25th noticeably decreased the total ^{15}N abundance in total N by 23.4 and 40.7 % in the upper leaf, by 15.5 and 32.7 % in the middle leaf, by 10.1 and 7.27 % in the lower leaf, and by 14.9 and 19.4 % in the stem, which resulted in a reduction of 15.1 and 19.1 % in shoots at the maturity stage of the FCT plants, respectively.

The ^{15}N use efficiency (^{15}NUE) in FCT shoots

The ^{15}NUE was calculated by ^{15}N tracing technology (Figure 2). Results revealed that postponing the transplanting date significantly improved ^{15}NUE at the rosette stage, but the different transplanting time had no notable effect on ^{15}NUE at the topping and maturity stage. Furthermore, the ^{15}NUE at the topping stage was 27.0 - 28.4 %, which was similar to that measured at the maturity stage (≈ 30 %). Therefore, it indicated that fertilizer-^{15}N was mainly taken up before topping and soil N was the main N source for FCT plants after topping.

Nicotine concentration in FCT shoots

Delaying the transplanting time decreased the nicotine concentration in tobacco leaves after topping (Table 4). For example, compared to the FCT seedlings transplanted on May 5th, delaying the transplanting time to May 15th and 25th, the nicotine concentrations in the FCT leaves noticeably decreased by 33.4 and 24.9 % in the upper leaf, by 26.8 and 30.6 % in the middle leaf, and by 24.3 and 35.8 % in the lower leaf at the maturity stage of the FCT plant.

Proportion of nicotine-N to total N in FCT shoots

Postponing the transplanting time decreased the proportion of nicotine-N to total N taken up by FCT plants at their topping and maturity stages (Table 5). For example, compared to the transplanting time of May 5th, postponing the transplanting date to May 15th and

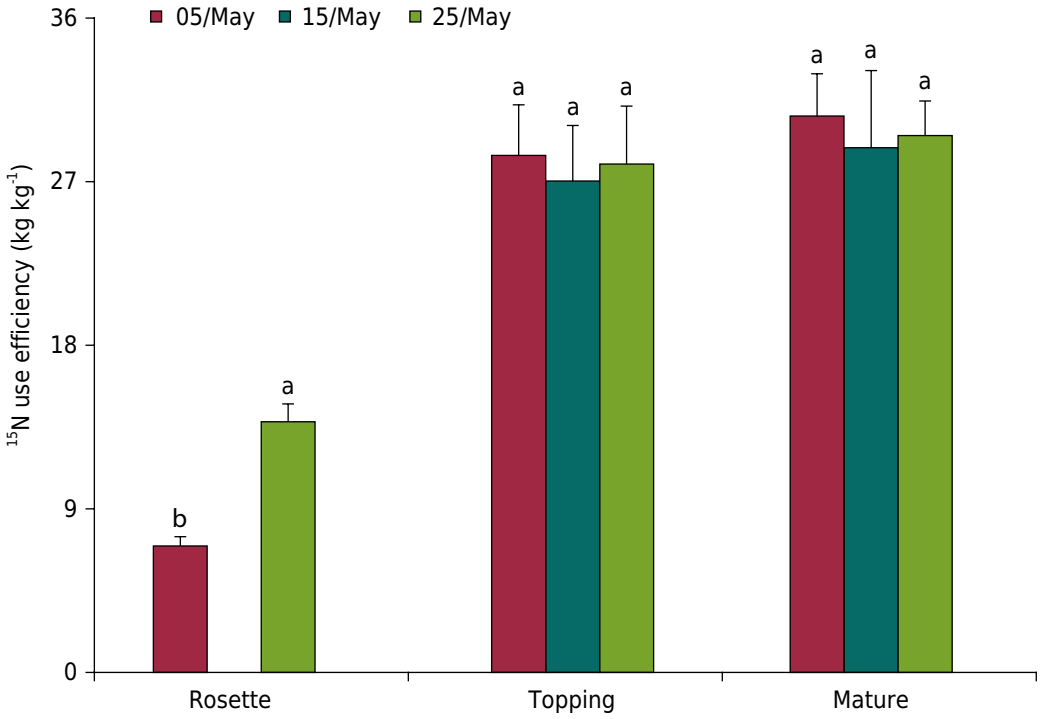

Figure 2. Effects of different transplanting time on ^{15}N use efficiency by FCT plants at different growing stages. The bars above the columns indicate the standard deviation (n =3). Different letters in the same color column indicate significant differences at the 0.05 level.

25th, the proportion of nicotine-N to total N of the FCT leaves decreased by 32.8 and 26.9 % in the upper leaf, by 46.0 and 50.6 % in the middle leaf, by 34.5 and 38.0 % in the lower leaf, and by 4.34 and 25.6 % in the stem, thus inducing a reduction of 35.1 and 33.5 % in shoots at the maturity stage, respectively.

The proportion of nicotine-^{15}N to total nicotine-N in FCT shoots

Delaying the transplanting time of FCT seedlings significantly decreased the nicotine-^{15}N abundance in total nicotine-N of shoots at the topping and maturity stages of FCT (Table 6). For example, compared to transplanting FCT seedlings on May 5th, delaying the transplanting time to May 15th and 25th decreased the nicotine-^{15}N in total nicotine-N

Table 4. Effects of different transplanting time on nicotine concentration of FCT shoots at different growing stages (%)

Organs	May 5th	May 15th	May 25th
	Nicotine concentration of FCT		
	%		
	Rosette stage		
Shoot	0.46±0.01b	-	0.59±0.05a
	Topping stage		
Upper leaf	0.30±0.03a	0.23±0.01ab	0.19±0.03b
Middle leaf	0.63±0.03a	0.42±0.04b	0.34±0.03c
Lower leaf	1.14±0.24a	0.58±0.08b	0.52±0.04b
Stem	0.43±0.01a	0.41±0.03a	0.40±0.01a
	Maturity stage		
Upper leaf	3.17±0.12a	2.11±0.10c	2.38±0.05b
Middle leaf	1.83±0.19a	1.34±0.08b	1.27±0.05b
Lower leaf	1.48±0.05a	1.12±0.07b	0.95±0.04c
Stem	0.45±0.05a	0.50±0.03a	0.42±0.06a

Values are means ± standard deviation (n =3). Means followed by different letters in the same row indicate significant difference at the 0.05 level. -: not tested.

Table 5. Effects of different transplanting time on proportion of nicotine-N to total N of FCT shoots at different growing stages (%)

Organ	May 5th	May 15th	May 25th
	Proportion of nicotine-N to total N of FCT		
	%		
	Rosette stage		
Shoot	2.44±0.10b	-	2.83±0.08a
	Topping stage		
Upper leaf	2.29±0.36a	2.02±0.38a	1.36±0.15b
Middle leaf	7.91±0.19a	4.36±0.66b	2.92±0.26c
Lower leaf	19.1±4.88a	5.50±0.98c	9.20±0.32b
Stem	6.81±0.26a	6.66±0.92a	4.26±0.20b
Shoot	6.80±0.63 a	4.43±0.10b	3.44±0.12c
	Maturity stage		
Upper leaf	26.8±2.48a	18.0±2.38c	19.6±2.84b
Middle leaf	23.9±2.63a	12.9±3.01b	11.8±4.83b
Lower leaf	25.8±5.94a	16.9±1.42b	16.0±078b
Stem	5.07±0.26a	4.85±0.27a	3.77±0.84b
Shoot	19.4±0.58a	12.6±0.38b	12.9±1.41b

Values are means ± standard deviation (n =3). Means followed by different letters in the same row indicate significant difference at the 0.05 level. -: not tested.

Table 6. Effects of different transplanting time on nicotine-^{15}N abundance in total nicotine-N of FCT shoots at different growing stages (%)

Organ	May 5th	May 15th	May 25th
	Nicotine-^{15}N abundance in total nicotine-N of FCT		
	%		
		Rosette stage	
Shoot	57.1±0.39b	-	69.7±2.88a
		Topping stage	
Upper leaf	51.2±1.62a	43.2±6.59b	35.3±4.14c
Middle leaf	54.4 ±1.53a	45.7±2.21b	34.6±3.56c
Lower leaf	60.0±2.38a	49.4±0.97b	49.1±1.05b
Stem	37.0±4.97b	39.5±1.08b	47.6±1.37a
Shoot	51.5±0.72a	44.2±1.21b	42.8±1.03b
		Maturity stage	
Upper leaf	29.7±4.96a	19.1±3.11c	23.8±3.30b
Middle leaf	32.0±1.67a	25.3±0.94b	27.5±2.00b
Lower leaf	49.3±0.57a	41.9±4.30b	45.7±1.34ab
Stem	28.8±1.52a	21.6±0.48b	21.3±1.34b
Shoot	32.5±3.19a	26.3±1.82b	24.2±2.65b

Values are means ± standard deviation (n =3). Means followed by different letters in the same row indicate significant difference at the 0.05 level. -: not tested.

by 35.7 and 19.9 % in the upper leaf, by 20.9 and 14.1 % in the middle leaf, by 15.0 and 7.30 % in the lower leaf, and by 25.0 and 26.0 % in the stem, thus leading to a decrease of 19.1 and 25.5 % in shoots at the maturity stage, respectively.

DISCUSSION

The dry matter and N accumulated in FCT plants during the growth period was mainly distributed in the upper leaf and stem, followed by the middle leaf and lower leaf (Table 2). Likely, this is mainly due to the N nutrient being easily remobilized in the tissues of the FCT and preferentially allocated to new active sites of the plant (Marschner, 1995).

Obviously, the transplanting time influenced the growth and development, as well as the yield and quality of FCT plants. The FCT seedlings transplanted early likely advanced the differentiation of flower buds, which induced early blossoming and thus decreased the leaf numbers and yield of the FCT plant, since the seedlings were in adverse conditions of low temperature and lack of sunlight during the early growth period. It has been proved that a lack of light decreases the thickness of palisade and spongy tissues, as well as the net photosynthetic rate and stomatal conductance of leaves, which results in a decrease in the thickness and dry matter weight of FCT leaves (Zheng et al., 2009; Wang et al., 2011). Our study also indicated that delaying the transplanting time noticeably increased the dry matter weight and N accumulation in the aboveground parts of FCT plants (Table 2).

Delaying the transplanting time decreased the proportion of fertilizer-^{15}N to total N (Table 3). It is well known that the mineralization of soil N is regulated by microbial communities, as well as their populations and activities, which are closely related to soil conditions such as temperature, water, and so forth. Within a certain range, the activities of soil microbes increased with rising temperature, which promoted the mineralization rate of soil N (Lewis and Thomas, 1982; Hagedorn et al., 1997). Furthermore, the growth and development of the plant root is also closely related to environmental conditions (Barnes, 2002; Jose et al., 2003). In this study, compared to the later transplanting dates (i.e., May 15th and 25th), the seedlings transplanted on May 5th were subjected to lower temperature

(<18 °C) and rainfall (<80 mm) in the early growth period (Figure 1), which stagnated the soil microbes, and thus lowered inorganic N and its availability (Markhart et al., 1979). However, the climatic conditions improved over time, which promoted the mineralization of soil N and increased available N in the soil around plant roots (Marchetti et al., 2006). Consequently, this was favorable to the growth and development of FCT roots, as well as ^{15}N uptake and accumulation (Thomsen et al., 2010; Rowe et al., 2012). However, exogenous mineral N decreases mainly due to plant acquisition, microbial immobilization, leaching, and denitrification. Thus, mineral N derived from soil organic matter (SOM) is increasingly important for plant nutrition throughout the crop cycle. At harvest, SOM is the main source of N for plants. This was also proved by the low NUE observed in this study (<30 %) (Figure 2). Results indicated the higher contribution from soil N than fertilizer N to the FCT shoots after topping (Xi et al., 2005).

Moreover, delaying the transplanting time decreased the nicotine concentration of FCT leaves (Table 4). It has been said that the transition from nitrate reduction metabolism to starch accumulation metabolism at the right moment is critical for the quality of tobacco leaves, and a sharp decrease in nitrate reductase (*NaR*) activity can be used as a clear single of the transition from N metabolism to carbon accumulation metabolism (Weybrew et al., 1983). Postponing the transplanting time noticeably decreased the activities of *NaR* and glutamine synthetase (GS) and increased the activity of amylase in leaves, thus moderating the C and N metabolisms in FCT plants (Guo, 2005). The dilution effect of dry matter may be another explanation for the nicotine reduction (William et al., 1989).

Although the amounts of N participating in nicotine synthesis increased with growth, especially after excising the apex of FCT plants (Zador and Jones, 1986), nicotine synthesis mainly relied on the N taken up by the root system before topping, which was then conveyed to the aboveground parts through the xylem (Yoshida and Takahashi, 1961). Therefore, the contribution of soil N to nicotine synthesis was higher than that of fertilizer N after topping (Xi et al., 2005). It is well known that nicotine concentration is a key index for evaluating the quality and industrial availability of tobacco leaves, and it is closely correlated with the amount of N supplied and taken up since N is 17.3 % of the molecular weight of nicotine (Collins and Hawks, 1993). Transplanting time significantly affects the individual development and quality of FCT plants mainly by its influence on the climatic conditions to which plants are subjected during the field growth period, which is one of the most important factors for producing sound tobacco (Patel et al., 1989; Biglouei et al., 2010; Alameda et al., 2012). Our study showed that postponing the transplanting time decreased the proportions of nicotine-N to total N and fertilizer-^{15}N to total nicotine-N in the aboveground parts of FCT (Table 5 and Table 6). This may be the reason for the reduction in nicotine concentration in FCT leaves. The FCT seedlings transplanted later likely were subjected to better climatic conditions, such as a more favorable temperature, soil moisture, and sunlight during the field growth period, which promoted photosynthesis and moderated the C and N metabolisms of FCT plants. In addition, more N participated in the synthesis of organisms (e.g., protein), thus increasing the dry matter weight of FCT and reducing the proportions of N for nicotine synthesis.

CONCLUSIONS

Postponing the transplanting time of FCT seedlings increased the dry matter and N uptake in shoots before topping, whereas it decreased the proportion of fertilizer N to total N and the proportion of total N and fertilizer-^{15}N for nicotine synthesis, which resulted in a reduction in the nicotine concentration in FCT leaves. Therefore, delaying transplanting time is advantageous in promoting ^{15}N use efficiency while reducing the nicotine content in leaves, which is imperative in improving the quality of FCT leaves for industrial availability. However, it is also imperative to conduct long-term field studies to further investigate the effect of transplanting time on the physiological traits of FCT plants (e.g., the antioxidant system, root activity, etc.) and soil biological properties.

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