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Comparison of N uptake and internal use efficiency in two tobacco varieties

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ABSTRACT

To explain the observation in field experiments that tobacco variety CB-1 was more nitrogen (N)-efficient than K326, the influence of two N levels on growth, N uptake and N flow within plants of the two tobacco varieties was studied. Xylem sap from the upper and lower leaves of both tobacco varieties cultured in quartz sand was collected by application of pressure to the root system. CB-1 took up more N with smaller roots at both high (HN, 10 mmol L^{-1}) and low (LN, 1 mmol L^{-1}) N levels, and built up more new tissues in upper leaves especially at LN level, than K326. Both varieties showed luxury N uptake, and CB-1 accumulated significantly less NO_3^- in new tissues than K326, when grown at the HN level. At both N levels, the amount of xylem-transported N and phloem-cycled N from shoot to root in K326 was greater than those in CB-1, indicating higher N use efficiency in CB-1 shoots than in K326 shoots. The major nitrogenous compound in the xylem sap was NO_3^- irrespective of N level and variety. Low N supply did not cause more NO_3^- reduction in the root. The results indicated that the N-efficient tobacco variety CB-1 was more efficient in both N uptake by smaller roots and N utilization in shoots, especially when grown at the LN level.

1. Introduction

Chemical nitrogen (N) fertilizer is the main source of nutrients applied to the soil for increasing crop yields in intensive agricultural systems. Chemical N fertilizer is one of the most energy-consuming nutrients, and is likely to cause environmental problems when incorrectly applied [1,2]. Improving nitrogen use efficiency (NUE) is an important task for both sustainable agriculture and global ecosystem stability [3,4]. Variation in N efficiency is known to exist among cereal genotypes, such as wheat (*Triticum aestivum* L.) [5,6], oat (*Avena sativa*) [7,8], rice (*Oryza sativa* L.) [9], and maize (*Zea mays* L.) [10,11]. With sufficient N supply, variation in NUE is due largely to differences in N uptake efficiency, whereas with deficient N supply, such variation is due mainly to differences in utilization of accumulated N [12]. To improve NUE, it is desirable to improve simultaneously both uptake efficiency and utilization efficiency of plants.

Cycling of mineral nutrients and carbon compounds between the root and shoot has been convincingly demonstrated [13–21]. Nitrogen transport and partitioning within plants vary among species, including maize [20], wheat [13,14], tobacco (*Nicotiana tabacum* L.) [22], and castor bean (*Ricinus communis* L.) [23]. Enhanced N cycling between the shoot and root under lower N supply has been reported [13,20,22–24]. Quantification

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of mineral fluxes in the xylem sap can be used for investigating mineral uptake and cycling within plants [25].

The flue-cured tobacco varieties CB-1 and K326 have been widely used in tobacco production in Fujian province in recent years. Regardless of different N application rates (98 kg ha^{-1} for CB-1 and 120 kg ha^{-1} for K326) in local tobacco production, the two varieties had similar leaf N concentrations and yields, indicating that CB-1 is more efficient in N use than K326 [26]. However, differences in N uptake and cycling in the varieties and the extent to which translocation in the xylem and N circulation are altered by different N supplies are not fully understood. The present study was performed to address these questions, with the aim of investigating the influence of N levels on growth, N uptake, and N cycling in the two tobacco varieties, and better understanding the mechanisms underlying the differences in NUE between the two varieties.

2. Materials and methods

2.1. Plant culture and growth conditions

Tobacco seeds (*N. tabacum* var. CB-1 and K326) were germinated in a mixture of 60% (v/v) peat culture substrate, 20% (v/v) vermiculite, and 20% (v/v) perlite, and grown in a seedbed in a naturally lit glasshouse for 40 days. Before they were transferred to 2.0 L pots (one plant per pot) containing quartz sand (0.25–0.50 mm in diameter), the tobacco seedlings were washed with tap water to remove all substrate from the roots. The plants were watered initially with a half-strength nutrient solution. After 1 week a full-strength nutrient solution was substituted, consisting of the following compounds (mmol L^{-1}): 2 NH_4NO_3, 1 KH_2PO_4, 2.5 K_2SO_4, 2 $MgSO_4{\cdot}7H_2O$, 5 $CaCl_2{\cdot}2H_2O$, 3.7×10^{-2} Fe-EDTA, 4.6×10^{-2} H_3BO_3, 7.65×10^{-4} $ZnSO_4{\cdot}7H_2O$, 3.2×10^{-4} $CuSO_4{\cdot}5H_2O$, 1.6×10^{-5} $(NH_4)_6Mo_7O_{24}$, and 9×10^{-3} $MnCl{\cdot}4H_2O$. The initial pH of the nutrient solution was adjusted to 6.0 ± 0.1. The plants were watered every 3 days before the beginning of the treatments and daily thereafter during the treatments in the morning with an excess of the nutrient solution. Small holes at the bottom of the pot allowed drainage. The drainage solution was discarded. The plants were grown under controlled conditions with a 14 h photoperiod. The photosynthetically active radiation at the surface of the pots was 210–250 μmol m^{-2} s^{-1} provided by reflector sunlight metal halide lamps (250 W, Philip Hipplus, Belgium).

2.2. Treatments and harvest procedures

The first harvest was performed 65 days after germination or 25 days after transfer to the controlled conditions, and the second harvest was performed 9 days later. For harvesting, the six plants of the two tobacco varieties each were divided into three groups of similar size and developmental stage. One group of each variety was used for the first harvest and the remaining two groups for the second harvest. On the day of the first harvest, the remaining two groups of plants were treated with either 1 mmol L^{-1} N (LN) or 10 mmol L^{-1} N(HN) as NH_4NO_3. The other components of the nutrient solution were as described above.

Leaves were numbered in ascending order, starting from the lowest mature leaf, which was designated as leaf 1. Smaller leaves that had already senesced were removed. The youngest unfolded leaf was leaf 8 at the first harvest; leaf 10 for the LN-treated plants and leaf 12 for the HN-treated plants at the second harvest, respectively. At harvest, plants were separated into roots, stem, lower leaves (1–6) and upper leaves (leaves 7–8 for the first harvest and 7–12 for the second harvest). Roots were washed free of sand with tap water. The two strata of leaves were divided into two lateral symmetrical parts: one was kept at −20 °C until analysis of tissue NO_3^- contents, and the other with roots and stem was treated at 105 °C for 30 min, dried at 70 °C to constant weight, weighed, and ground into powder.

Appropriate amounts of the ground plant tissues were used to determine total N content by a modified Kjeldahl digestion method that included reduced nitrate [27]. Calcium in the tissue was analyzed using a flame spectrophotometer (Cole-Parmer 2655-00, Cola-Paymqv Company, USA). To measure tissue NO_3^- content, the frozen leaves were homogenized with distilled water and centrifuged. The extracts of the leaf samples were subjected to NO_3^- determination by a modified salicylic acid method [28].

2.3. Collection of xylem sap

For collection of xylem sap, plants were grown in special pots in order to apply pressure to the root system [29], but treated in the same way as the plants for harvest. The procedure for collection of xylem sap was described by Jeschke and Pate [15]. Briefly, xylem sap was collected by compressing the moist quartz sand substrate and the root system in a pressure vessel. At approximately midway along the length of leaves an incision was made into the midrib. The cut surface was carefully washed and a Teflon tube was attached. After slow application of pressure, xylem sap started to exude from the midribs after a balancing pressure was reached [29], and the sap was collected 50 kPa above this pressure. The first exudate was discarded to avoid contamination from cut cells. Xylem sap was kept on ice during collection and stored at −20 °C before analysis. Samples were taken from leaves 5 and 7. Xylem sap collection was repeated three times 2, 5, and 8 days after commencement of the treatments. Calcium in the xylem sap was analyzed directly after appropriate dilution using ICP (Perkin Elmer 3300 DV, USA). Nitrate and ammonium in the xylem sap were analyzed following dilution by a TRAACS-2000 auto-analyzer (Bran + Luebee, Germany). Amino acids in the xylem sap were determined using an amino acid autoanalyzer (Hitachi, 8800, Japan). The total N in the xylem sap was the sum of measured NO_3^- N, NH_4^+ N, and amino acid N.

2.4. Estimation of net N flow through the xylem and phloem in the whole plant

Net N flow in plants was estimated using the method described by Armstrong and Kirkby [30] and Jiang et al. [18]. The assumption of the model is that nutrients were transported solely through xylem and phloem, while Ca^{2+} can be transported only apically through xylem and has no mobility in phloem.

2.5. Statistical treatment

Dry weight (DW) and total N increases were obtained from six replicates of each treatment at the first and second harvests. All further analyses were performed with six individual samples for each organ. For statistical analysis, the SAS program for Windows (version 6.12) was used (SAS 1987). Differences between means of all the parameters were tested with one-way (initial value) and two-way (net increase in the second harvest) analyses of variance (ANOVA).

3. Results

3.1. Plant growth

At the first harvest, the DW of individual organs and whole plants of the two tobacco varieties was not significantly different. After 9 days of growth under LN (1 mmol L^{-1}), the plants of the two varieties showed N deficiency symptoms including fewer leaf numbers, light green leaves, and early senescence of lower leaves, especially for K326, compared with those grown at HN (10 mmol L^{-1}) (data not shown). In comparison with CB-1, young leaf growth (upper leaves) of K326 was inhibited by LN (Table 1). The upper leaves contributed most to total DW gain in both varieties, irrespective of N level. The root DW increase of CB-1 was less than that of K326 (Table 1), and thus the whole root DW of CB-1 was significantly lower than that of K326 after 9 days of growth.

3.2. Tissue N and NO_3^- contents

At the first harvest, the N contents of upper leaves, shoots, and whole plants in K326 were significantly higher than those in the CB-1 counterparts. After 9 days of growth at both N levels, however, the difference in N content between the two varieties was not significant (Table 2). Although total net DW gain of HN-supplied plants was only slightly higher than that of LN-supplied plants (Table 1) after 9 days of treatment, the net N increase of HN-supplied plants was significantly higher than that of LN-supplied plants in both varieties. At both N levels the greatest net increase of N was found in the upper leaves. There was even net N export from the lower leaves in both varieties when they were grown under LN (Table 2).

NO_3^- contents in the two strata of leaves of both tobacco varieties grown at LN were very low, and there were no differences in leaf NO_3^- contents between the two varieties. Increased N supply in the growth medium caused dramatic increases in leaf NO_3^- contents in both varieties, especially in the upper leaves. More NO_3^- was accumulated in the upper leaves of K326 than in those of CB-1 grown at the HN level (Fig. 1).

3.3. Nitrogenous compounds in xylem sap

Total N concentration of xylem sap was higher when plants grew under HN, and total N concentration was lower when plants grew under LN, irrespective of leaf position. In all cases, NH_4^+-N concentration was very low. NO_3^- N was the major nitrogenous compound in xylem sap, especially when LN was supplied. The proportion of NO_3^- N to total N measured in xylem sap was higher in plants grown under LN than under HN (Table 3).

3.4. Estimation of net N flow within plants

In all cases, the upper leaves of the treated plants were the main sink for N deposition and accounted for 61%, 84%, 67%, and 79% of the total N taken up in CB-1-HN, CB-1-LN, K326-HN, and K326-LN, respectively. Given that the amount of xylem-transported N (equal to the sum of N import into different shoot tissues) exceeded the total N uptake, phloem re-translocation of N from shoot to root contributed to the xylem-transported N. The amount of N re-translocated in the phloem constituted 19% and 25% of N transported in the xylem of CB-1 and K326 grown under HN, and 36% and 51% under LN, respectively. The N recycled in phloem from shoot to root came from different leaves (Fig. 2).

4. Discussion

4.1. Effects of nitrogen application rate on plant growth and N uptake

At the first harvest, the N content was significantly higher in K326 than in CB-1 whole plants. However, whole plant DWs of the two varieties were not significantly different (Tables 1 and 2). The results indicated that NUE, defined as plant biomass production on the basis of plant N, was higher in CB-1 than in

Table 1 – Initial values and net increases in DW of different organs and of whole plant of tobacco varieties CB-1 and K326 grown under HN (10 mmol L^{-1}) and LN (1 mmol L^{-1}) conditions over a 9-day study period.

Treatment	Variety	Dry weight (g per plant)					
		Upper leaf	Lower leaf	Stem	Root	Shoot	Whole plant
Initial DW							
	CB-1	0.49 a	1.64 a	0.39 a	0.59 a	2.52 a	3.11 a
	K326	0.59 a	1.63 a	0.41 a	0.52 a	2.63 a	3.15 a
Net increases at the second harvest							
HN	CB-1	3.70 a	1.53 a	1.09 ab	0.61 b	6.32 a	6.93 ab
	K326	3.86 a	1.12 bc	1.21 a	0.83 a	6.19 a	7.02 a
LN	CB-1	3.37 ab	1.01 c	0.89 b	0.67 b	5.28 b	5.95 b
	K326	2.88 b	1.84 a	0.91 b	0.82 a	5.63 ab	6.45 ab

Values in each column followed by the same letters for initial values and the second harvest are not significantly different at $P \leq 0.05$.

Table 2 – Initial values and net increases in N contents of different organs and of whole plant of tobacco varieties CB-1 and K326 grown under HN (10 mmol L^{-1}) and LN conditions (1 mmol L^{-1}) over a 9-day study period.

Treatment	Variety	N content (mmol per plant)					
		Upper leaf	Lower leaf	Stem	Roots	Shoot	Whole plant
Initial value							
	CB-1	1.78 b	3.96 a	0.37 a	1.05 a	6.11 b	7.16 b
	K326	2.19 a	4.07 a	0.40 a	1.09 a	6.66 a	7.75 a
Net increase at the second harvest							
HN	CB-1	15.36 a	5.92 a	1.87 a	1.99 a	23.15 a	25.13 a
	K326	16.34 a	4.22 b	2.08 a	1.93 a	22.63 a	24.56 a
LN	CB-1	4.85 b	−0.81 c	0.65 b	1.07 b	4.68 b	5.75 b
	K326	4.21 b	−0.50 c	0.56 b	1.06 b	4.27 b	5.33 b

Values in each column followed by the same letters for initial values and the second harvest are not significantly different at $P \leq 0.05$.

K326. New tissues (upper leaves) were the main sink for both assimilates and N deposition, despite the N levels in the present study. In comparison with LN treatment, the NUE in both varieties grown at the HN level was very low. This low efficiency was due to luxury N uptake by plants grown at the HN level, as indicated by the large amount of NO_3^- accumulating in leaf tissues, especially in K326 (Fig. 1). The results indicated that CB-1 was more efficient in N uptake and building new tissue than K326, especially when they were grown under LN. This inference was confirmed by N uptake rate expressed relative to unit root DW. The N uptake rates of CB-1 and K326 were 20.9 and 18.2 mmol L^{-1} g^{-1} DW root 9-day^{-1}, respectively, when they were grown under HN, and 4.6 and 4.0 when grown under LN (calculated based on the results in Tables 1 and 2). In comparison with the growth at the HN level, more DW was formed in the upper leaves of CB-1 than in K326 only after a 9-day decrease in LN supply. The results indicated that CB-1 is more tolerant to LN and more efficient in both N uptake and utilization than K326.

4.2. Flow and partitioning of N within plants

There was no significant difference in the amount of N taken up by the two tobacco varieties at both N levels. However, CB-1 had smaller roots than K326 (Tables 1 and 2). Nitrogen uptake efficiency depends on root size and uptake ability [31,32]. A demand-driven regulatory mechanism of N uptake has been described [20,33,34]. Although the amount of xylem-transported N was greater in K326 than in CB-1, less N was re-translocated from the shoot to the root in CB-1 than in K326, irrespective of N level (Fig. 2), indicating a higher NUE in CB-1 shoots than in K326 shoots. The importance of nutrient cycling within plants for signaling shoot demand for nutrients has been described by Marschner et al. [35]. Reduced translocation of N from the shoot to the root acts as an important signal of feedback control and stimulates more N uptake by roots [36].

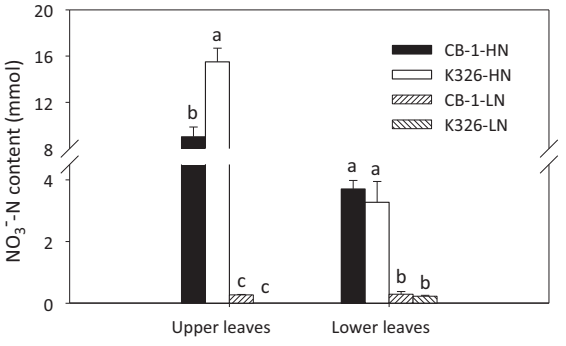

Fig. 1 – NO_3^- content in upper and lower leaves of the two tobacco varieties CB-1 and K326 after 9 days of growth under HN (10 mmol L^{-1}) and LN (1 mmol L^{-1}). Different letters above columns within each leaf stratum denote significant differences at $P \leq 0.05$.

Total N transported in the xylem and cycled in the phloem in the two tobacco varieties was significantly lower under LN than under HN (Table 3, Fig. 2). In contrast, transported K^+ in the xylem of tobacco plants grown at high nutrient levels was almost the same as that grown at low nutrient levels, and the same was true for cycled K^+ in the phloem [19]. Unlike K, N is incorporated into plant tissues, and thus the amounts of free N cycled in phloem and transported in xylem of plants grown under LN should be lower than that of plants grown under HN. The observation that almost no nitrate could be detected in leaf tissues at the LN level implies that most deposited N in leaves was incorporated into leaf tissues (Fig. 1). In all cases, independent of variety and N level, the amount of N transported in the xylem was far more than that taken up during the same period, so that the excess N in the xylem must have been compensated from the phloem (Fig. 2). The amount of N re-translocated in the phloem contributed respectively 19% and 25% of the xylem transported N in CB-1 and K326 grown under HN and 33% and 51% under LN. In comparison with plants grown under HN, the proportion of N translocation from the phloem to the xylem at LN level increased. Increased N recycling from the shoot to the root is important for the nutrient demands of root growth and for providing the driving force for long-distance solute transport, especially when plants are grown under nutrient limited conditions [35]. The phloem-re-translocated N from the shoot to the root came from the leaves. Net N efflux from the lower leaves occurred in both varieties grown under LN (Table 2, Fig. 2). Under LN, NUE depends on N remobilization and utilization of accumulated N within plants [12,37].

Low N supply shifted nitrate reduction towards the root [22,38]. In maize, the major nitrogenous compound detected in the xylem sap of different leaves was nitrate when plants were

Table 3 – Concentrations of different nitrogenous compounds in the xylem sap of upper and lower leaves of two tobacco varieties grown under HN (10 mmol L^{-1}) and LN (1 mmol L^{-1}) conditions over a 9-day study period.

	Upper leaf				Lower leaf			
	HN		LN		HN		LN	
	CB-1	K326	CB-1	K326	CB-1	K326	CB-1	K326
NO_3^- N	6.36 a[a]	5.41 a	1.80 a	3.11 a	6.16 a[a]	5.41 a	2.18 b	3.32 a
	(70.7)	(72.8)	(82.6)	(81.8)	(80.3)	(71.5)	(82.7)	(89.1)
NH_4^+ N	0.81 a[a]	0.63 a[a]	0.06 a	0.11 a	0.49 a[a]	0.58 a[a]	0.05 a	0.06 a
	(8.5)	(10.1)	(2.8)	(3.0)	(6.4)	(7.9)	(1.7)	(1.7)
Amino N	1.85 a[a]	1.17 a	0.32 a	0.58 a	1.02 a[a]	1.61 a	0.43 a	0.35 a
	(20.8)	(17.1)	(14.6)	(15.2)	(13.3)	(20.6)	(15.6)	(9.3)

Values in parentheses indicate the proportions of the nitrogen forms to total N measured in the xylem sap.
Values in each row under the same N level of the same leaf stratum followed by the same letter are not significantly different at $P \leq 0.05$.
[a] Difference between the HN and LN treatments is significant at $P \leq 0.05$ for the same leaf stratum of the same tobacco variety.

grown under HN, but amino acid N under LN [20]. This result was not observed in the present study. The major nitrogenous compound in the xylem sap of the upper and lower leaves of both tobacco varieties was nitrate, and the ratio of nitrate to total N in the xylem sap of different leaves even increased when both varieties were grown under LN (Table 3).

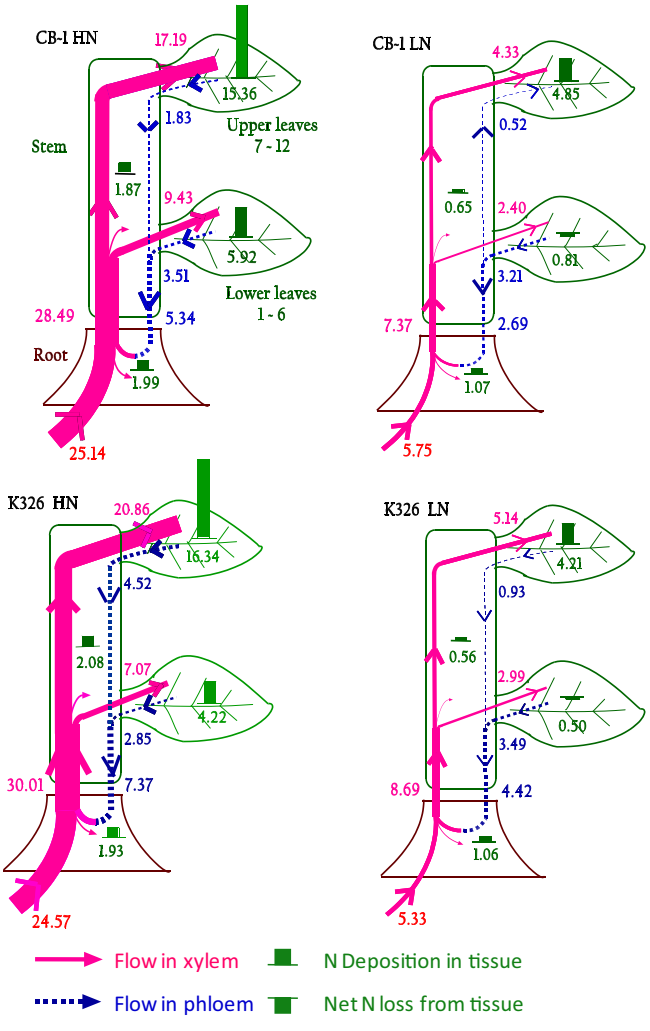

Fig. 2 – Flow profiles for uptake, transport, and utilization of N in two tobacco varieties, CB-1 and K326, grown under HN (10 mmol L^{-1}) and LN (1 mmol L^{-1}) over a 9-day study period. The values of N deposition and the statistical significance are given in Table 2. The width of lines and the heights of histograms are drawn in proportion to the net flow and deposition of N. The numbers indicate the amounts of uptake, transport, and utilization (mmol L^{-1} N $plant^{-1}$ over the 9-day study period).

The results from the present study indicate that CB-1 is more efficient in both N uptake by smaller roots and N utilization within plants, especially when grown under LN condition. These results were consistent with previous results [26] and could explain the observation that a similar yield of tobacco leaves can be achieved by lower chemical N fertilizer application to CB-1 than to K326.

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