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담배잎에서 칼륨과 능금산이 질산태질소의 이동 및 환원에 미치는 영향

이 윤 환

한국인삼연초연구소

ROLE OF POTASSIUM AND MALIC ACID FOR NITRATE TRANSLOCATION AND REDUCTION IN TOBACCO LEAF

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초 록

뿌리에서 흡수된 질소는 대부분이 질산태이온으로 잎의 엽맥까지 도달하나 엽육에서 급격히 환원되어 유기태질소로 동화한다.

잎의 주맥을 통과하여 세맥까지 질산태질소로 이동되며 전질소의 1／2 이상의 양까지 다다르나 엽육에서는 전질소함량이 엽맥의 5 배까지 증가되어도 질산태질소는 10^{-2} 정도로 급격히 환원되었다. 칼륨은 엽맥까지 질소와 동반 이동하나 엽육에서의 질산태질소환원에 의하여 이동이 차단되는 현상을 보였다.

엽육에 축적된 칼륨은 능금산의 축적을 촉진하였고 질산환원효소의 활성이 왕성하게 일어나는 하위엽에 높은 농도로 축적되었다.

INTRODUCTION

Nitrate is the dominant anion absorbed by plant (19, 23) and very impotant nutrient to be assimilated to protein. This ion accompanies with cations to sustain ionic balance (9, 13, 14, 15). Among the cations, potassium ion has effect on increasing nitrate uptake, translocation and promotion of nitrate reduction in the plant tissues (3, 18). Absorbed nitrate might be reduced to assimilate organic nitrogen compound. The negative charge from nitrate ion

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should be transferred to form of organic anions in plants (2, 15).

Malate is principal balancing acid in the metabolism of nitrate reduction to sustain the stoichiometry by synthesis and degradation to carbonate ion (20). The charge from originating in reduction of nitrate thus transferred to the nutrient medium as bicarbonate anion. By excretion of every bicarbonate, nitrate can be taken from the nutrient solution balanced with potassium ion already in the root. And the cycle can be repeated. Nitrate-malate shuttle scheme were proposed by Benzioni-Dijkshoorn (2, 8).

Tobacco plants prefer to absorb nitrate rather than ammonium. Cation uptake were promoted to maintain the ion balance at nitrate medium (25) and nitrate reduction was stimulated (26, 27). By these results, nitrate medium exceed ammonium in growth of tabacco plant (21).

Tobacco plant differentiated clearly to the distinguished tissues (10). Leaf specializes to the lamina as assimilation tissue and veins as water and nutrient conducting tissue. Passing through the veins, they were transported to the mesophyll and from which synthesized derivatives were conducted toward another tissues (11). And leaves at different stalk positions have distinct composition of compound during growing stage.

Following experiment investigated to provide information on the involvement of nitrate, potassium and malate accumulation and translocation at different tissues of leaves and compared the nitrate reduction of different stalks.

MATERIALS AND METHODS

8-leaf tobacco seedlings "Cultivar NC 2326" grown at seeding and seedling beds in the green house were transplanted to the field covered with polyethylene film at April 18. One hundred and twenty five kg/ha of nitrogen and phosphorus were applied as ammonium nitrate and triple super phosphate. Potassium was applied as potassium sulfate at rate of none and 250kg/ha. All fertilizers were rawcast and incorporated into soil to a depth of 10-15 cm with 12 M/T of rice straw compost at 10 days before transplanting.

Tobacco plants were harvested within the vigorous growing stage from mid of May to beginning of June. Plants grown uniformly were selected randomly. Leaves were removed at 10 O'clock of sun-shine days and put them into stylenepolymer foaming box with ice. The box was carried to lab-room conditioned temperature under 15°C. Leaves were seperated into midvein and lamina, sliced into narrow pieces and stored under −20°C at refrigerator for analysis of nitrate reductase activity and organic acid. Remains of slices were dried at electric wave dry oven quickly for nitrate and inorganic analysis.

Nitrate-N was determined with specific ionic meter (1) and total-N was distilled from the solution decomposed with sulfuric-salycilic acid solution.

Potassium concentration of digestion solution were determined by atomic absorption spectrophotometer.

The enzyme activity of nitrate reductase was modified the method 'in vitro assay' of Deane-Drummond and Clarkson (7).

Malic acid analysis were performed on Varian Model 3700 Gas Chromatograph equipped with FID detector. Column employed was packed with 5% SILAR 10°C 100/200 mesh (6).

RESULTS AND DISCUSSION

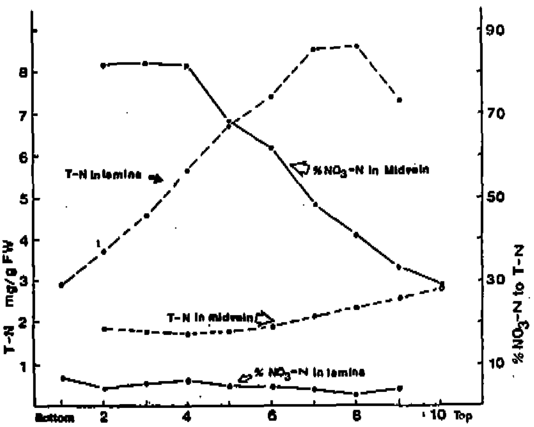

Fig. 1. Total nitrogen concentration and percentage of nitrate form in midvein and lamina at different stalk position of 13-leaf plant (Var. NC2326)

Total nitrogen of laminar and midvein showed very different patterns along the upper stalk position (Fig. 1). Those in lamina increased very rapidly until seventh leaf toward top, but those of midvein did flatly. Difference between lamina and veins were larger at upper stalk, and reached about three times of midvein at same position.

Nitrate nitrogen percentage in midvein exceeded over 80% at the lower stalk and dropped suddenly along the upper stalk position, but it remained almost 30% at top stalk.

In lamina, the percentage of nitrate-nitrogen didn't showed fluctuation under below 10%. It would be lower if lateral and minor veins were seperated from the lamina. These results suggested that most of nitrate absorbed from the root, reached to the veins of leaf. On the process of nitrate translocation through the diverged veins, it was distributed to the mesophyll cell and reduced gradually to organic nitrogen compounds (17).

Leaf veins are vascular tissues conducting water and nutrient to mesophyll cells. Midvein had large part of nitrate nitrogen not yet assimilated, and there were not significant differences among the parts of leaf on the way to move toward the leaf tip (Fig. 2).

But lateral veins diverged from the midvein had less portion of nitrate than midvein. On the way of nitrate translocation in the midvein along the axis, large parts of nitrate were diverged and a little remains were reached to the tip.

In lamina, nitrate content was too invisible by contrast with that in veins. It presumed that nitrate were reduced as soon as it reached to mesophyll.

Nitrate reductase activity was very high level at three bottom leaves and decreased apparently along the upper stalk position (Fig. 3). These activity levels of each stalks were consistent with the nitrate levels of midvein at same stalk.

These results expected that nitrate reductase activity was induced in proportion to the content of nitrate in midvein, and organic nitrogen compound being reduced at lower leaves would be translocated to the merismatic tissues of upper

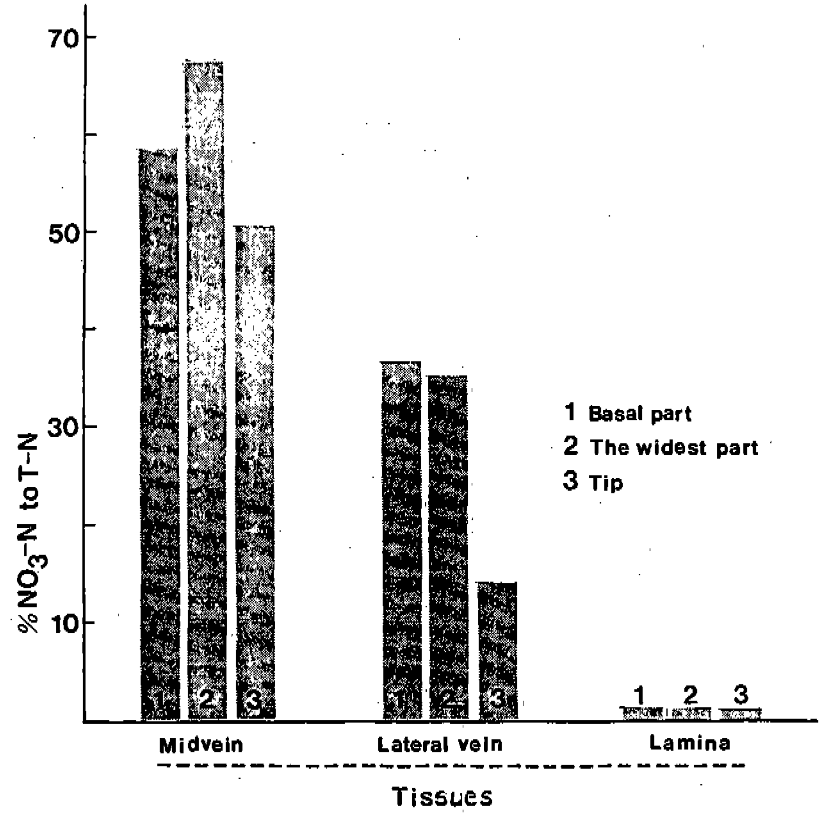

Fig. 2. Percentage of nitrate nitrogen in different tissues at three parts of the largest leaf at budding stage.

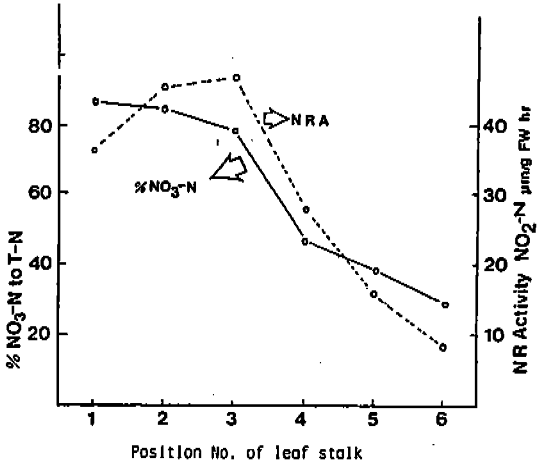

Fig. 3. Comparison between the nitrate nitrogen percentage in midvein and nitrate reductase activity (NRA) in lamina at the same stalk positions of 9-leaf plant.

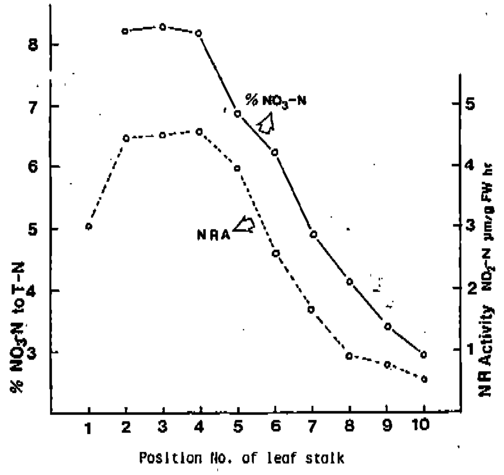

Fig. 4. Comparison between the nitrate nitrogen percentage in midvein and nitrate reductase activity (NRA) in laminae at the same stalk positions of 14-leaf plant.

leaves for leaf growth (22, 24).

At 14 leaf-plant (Fig. 4), same kind of relations between NR activity and nitrate content were found. But levels of NR activity were one tenth compared with 9 leaf-plant. It suggested that decrease of NRA by advancing growth stage was affected by the supply capability of organic nitrogen compound to the upper growing tissues. The plant having many leaves expanded extremely at bottom could supply assimilated organic nitrogen compounds from the wide unit of leaf area, but earlier stage of plant had narrow unit to supply for the demand of structure development of growing stage tissues (17).

Potassium content in midvein were much higher than in lamina at all stalk positions (Fig. 5). These results supported the view that potassium were blocked transferring to lamina because of exchanging counter ion by nitrate reduction.

Differences of potassium content from bottom to upper stalk position were less than nitrate, and more amounts of potassium were accumulated than nitrate at lamina. These data imply that much portion of potassium were bound with another constituents and transferred to compose the structure of organelles toward lamina and upper growing tissues (12).

For observation of accumulation and distribution of potassium in leaf tissues, tobacco plants were selected from the field applied with and without potassium. The content of potassium in basal and distal half showed in figure 6 and 7. We could find the differences of potassium content between lower and upper leaves. Lower leaves showed higher content of potassium than upper leaves. The vascular tissues, mid and lateral veins, accumulated higher content than lamina (16).

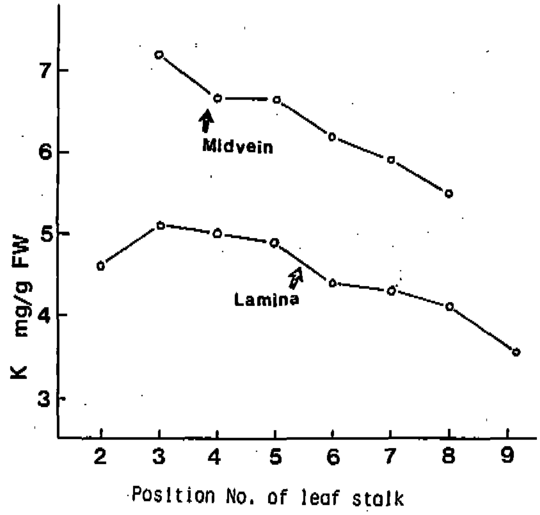

Fig. 5. Potassium concentration in midvein and lamina of leaves from different stalk positions.

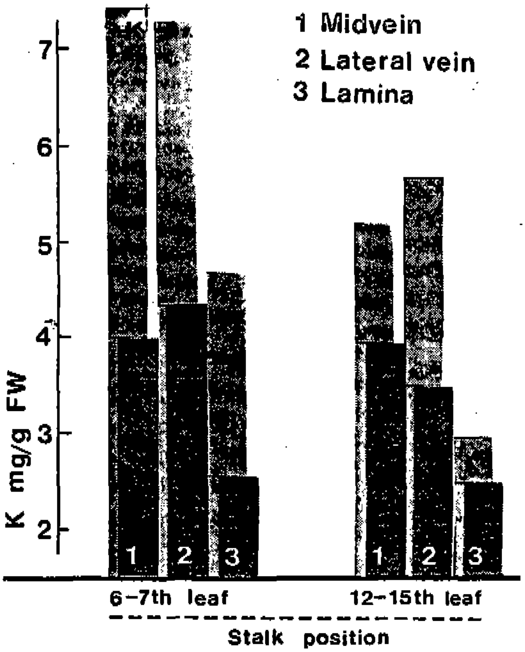

Fig. 6. Potassium concentration in different tissues at the basal half of leaves at 19-leaf stage with or without potassium application.

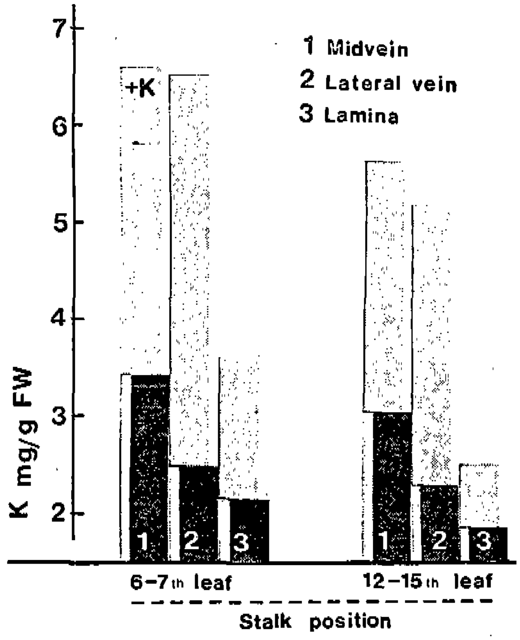

Fig. 7. Potassium concentration in different tissues at the distal half of leaves at 19-leaf stage with or without potassium application.

Basal half of leaves near to stem accumulated more potassium than distal half. These data suggested possibility of an association of potassium and nitrate ion during translocation along the vascular tissues. On the way of translocation through the vascular tissues, nitrate would be distributed to the mesophyll near veins, and content of nitrate gradually decreased until arrival to leaf tip. Potassium accompanied by nitrate would be isolated by reduction of nitrate and might be exchanged with another anions, also blocked translocation at tissues where nitrate reduced. Potassium concentration of non-applied plant was very little, but distribution in each tissues had similar trends as compared with applied plant.

The important results were found that non-applied treatment hadn't remarkable differences between lower and upper leaves. These meant that potassium for another organelles constituents might be required to sustained for another metabolism in plant (12).

Potassium content at upper stalk positions exceeded nitrate in proportion (Fig. 8). At 9-leaf stage, the proportion of potassium to nitrate were maintained constantly until third stalks from bottom, but from the fouth leaf, greater potassium accumulated as compared with nitrate. At 14-leaf plant, the proportion of potassium were going to turn over along the upper stalk position. These results predict that bound potassium with assimilation compounds translocated toward the top of growth tissues (4)

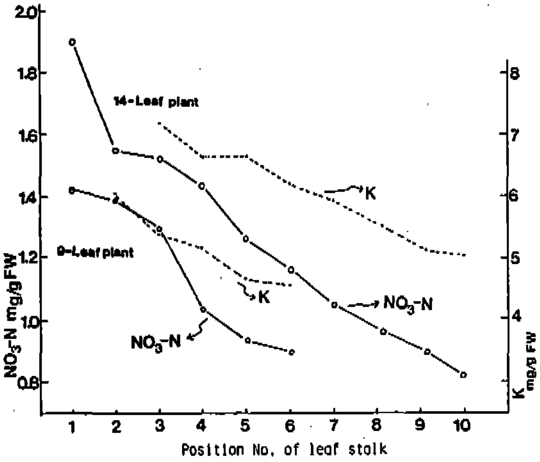

Fig. 8. Content of potassium and nitrate-N in midvein at different stalk position of 9-leaf plant and 14-leaf plant.

Higher content of malic acid were accumlated remarkably in lamina of lower leaves without the differences between basal and dital half (Fig. 9). Lamina of upper leaves had very low

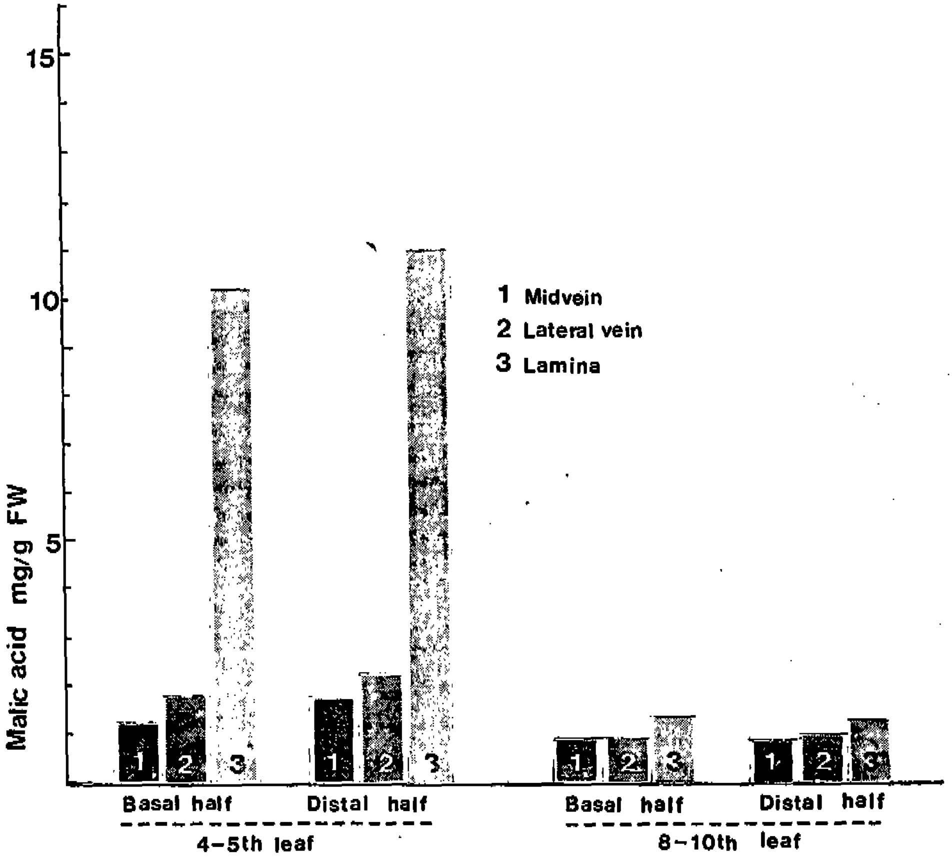

Fig. 9. Malic acid content in leaf tissues from different stalk positions at 14-leaf stage.

content of malic acid compared with lower leaves. This result implies that nitrate-counter anion of potassium required compensation of electric charge with malate at the tissues arising reduction of nitrate (5).

Potassium and nitrate content of leaf tissues at lower and upper stalk position had same trends with previous results (Tab. 1).

Nitrate, however, wasn't affected by the potassium application. Many papers reported that limiting uptake of potassium restricted severely nitrate translocation in the tissues. But these results were not consistent with the previous reports. We could suggest that soil would supply the potassium at least for sustaining translocation of nitrate in plants.

Nitrate reductase activity in lamina applied with potassium was higher than at non-applied treatment, and lower leaf had higher activity than upper leaves. This results could be explained by the phenomena that accumulation of nitrate at lower leaves promoted the induction of nitrate reductase activity.

Malic acid contents in the tissues of non-potassium treatment were lower than of potassium treatment, lamina of lower leaves con-

Table 1. Contents of potassium, nitrate nitrogen, malic acid and nitrate reductase activity (NRA) in leaf tissues of 19-leaf plant with or without potassium application.

	K	6-7th leaf		12-15th leaf	
		Lamina	Midvein	Lamina	Midvein
K	Applied	5.10 ± 0.26	6.72 ± 0.20	6.32 ± 0.18	6.00 ± 0.22
(mg/g FW)	Non-applied	3.44 ± 0.11	4.34 ± 0.49	3.02 ± 0.10	4.32 ± 0.23
NO_3-N	Applied	0.20 ± 0.02	1.42 ± 0.04	0.22 ± 0.01	0.93 ± 0.05
(mg/g FW)	Non-applied	0.21 ± 0.01	1.37 ± 0.04	0.24 ± 0.02	0.85 ± 0.04
Malic acid	Applied	9.88 ± 0.44	1.63 ± 0.21	2.03 ± 0.11	1.28 ± 0.18
(mg/g FW)	Non-applied	7.43 ± 0.25	2.04 ± 0.17	1.43 ± 0.13	1.32 ± 0.16
NRA	Applied	4.68 ± 0.26		2.47 ± 0.28	
(NO_2 μm/g FW. hr.)	Non-applied	1.08 ± 0.16		0.62 ± 0.10	

Values are mean ± standard error (n=5)

Treatment effects differ significantly within K, NRA ($p < 0.01$) and malic acid ($p < 0.05$).

tained higher concentration of malic acid than upper leaves. These data were consistent that the accumulation of potassium at midvein of lower leaves might be required the compensation of another anions by active reduction of nitrate at same tissues.

CONCLUSION:

Most of nitrogen absorbed from root reached to leaf vein as nitrate form and assimilated to organic nitrogen at lamina. Nitrate was main translocation form through midvein to diverged veins.

Over the half of total nitrogen in veins was nitrate but it was below one percent in lamina, in which total nitrogen was five times higher than vein.

Potassium was translocated through midvein accompanying with nitrate and accumulated at leaf veins due to interception of translocation by reduction of nitrate. Nitrate reductase activity increases with the increase of nitrate concentration at low leaves.

Potassium accumulated in the veins of lower leaves promoted the malic acid concentration for sustaining ionic balance.

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