# Relation Between Alkaloid Synthesis and Nutrient Absorption in Tobacco Plant

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## Incorporation process of nitrogen into alkaloid

Absorption and translocation of nitrogen in the tobacco plant wield great influences on the alkaloid synthesis because the alkaloid is a compound containing nitrogen. Alkaloid of tobacco plant accumulates mainly during the ripening period, but the nitrogen is absorbed principally before the ripening period. It is, therefore, significant that the alkaloid is synthesized using the nitrogen absorbed before the ripening period.

Nicotine which is an essential alkaloid in the tobacco plant used in this experiment is synthesized in the root. Nitrogen for nicotine synthesis would have to be supplied from the other parts of plant to the root during the ripening period.

**Table 1. Distribution of nitrogen among various parts of the tobacco plant during the ripening period (mg/plant)**

| Plant parts | Days after topping | | |
|---|---|---|---|
| | 0 | 17 | 30 |
| Leaves | 1920 | 1507 | 1483 |
| Stem | 853 | 1021 | 1245 |
| Root | 189 | 406 | 462 |

Distribution of nitrogen in the tobacco plant during growth was determined in detail. It was indicated that nitrogen was translocated from the leaves to the stem and the root during the ripening period.

Nitrogen translocated to the root would be used for nicotine synthesis. In order to ascertain this possibility, $^{15}N$-labeled nitrogen was injected into the tobacco leaves, and the distribution of $^{15}N$-labeled nitrogen in the different parts of the plant and the incorporation of $^{15}N$-labeled nitrogen into the nicotine were determined. It was indicated that $^{15}N$-labeled nitrogen was translocated into the root and incorporated into the nicotine and translocated to the leaves.

**Table 2. $^{15}N$-labeled total and alkaloid nitrogen in the plant which was cultured 14 days after injection of labeled nitrate into the middle leaves (mg/plant)**

| Plant parts | $^{15}N$-Total-N | $^{15}N$-Alkaloid-N |
|---|---|---|
| Upper leaves | 10.2 | 2.15 |
| Middle leaves | 13.9 | 1.54 |
| Lower leaves | 2.1 | 0.91 |
| Stem | 21.5 | 2.02 |
| Root | 13.2 | 1.19 |
| Total | 60.9 | 7.81 |

If nitrogen supply was stopped during the ripening period in the water culture experiment, translocation of nitrogen into the root from the leaves and increased accumulation of alkaloid were obtained. Before the ripening period, labeled nitrogen was supplied at the different stages and the plants were harvested at the ripening period. Incorporation rates of $^{15}N$-labeled nitrogen into the alkaloid were almost the same among the stages; that is, nitrogen is incorporated equally into the alkaloid

**Table 3. Incorporation rates of nitrogen into alkaloid absorbed during and before the ripening periods**

| | Total-N mg/plant (A) | Alkaloid-N mg/plant (B) | 100×B/A |
|---|---|---|---|
| Nitrogen absorbed before ripening period | 959.3 | 85.4 | 8.9 |
| Nitrogen absorbed during ripening period* | 751.6 | 113.4 | 15.1 |

* Labeled with $^{15}N$

when it is absorbed at any time before the ripening period.

When nitrogen was supplied during the ripening period, incorporation of nitrogen into the alkaloid was higher than that of the nitrogen absorbed before the ripening period. Absorption of nitrogen during the ripening period lowers not only the leaf quality but also increases excessively the alkaloid content in the leaves.

## Effect of nitrogen nutrition

In order to obtain sufficient yield of tobacco leaves, greater amount of nitrogen fertilizer would be supplied. But alkaloid content is increased with the supply of higher nitrogen level. Tobacco taste will soon become milder, therefore, the amount of nitrogen fertilizer has been controlled in Japan. Increased content of alkaloid at higher level of nitrogen supply is attributed to the increased amount of nitrogen used for alkaloid synthesis, residual absorption of nitrogen until the ripening period, and delayed harvesting which contributes to the longer period of alkaloid accumulation.

Reciprocal labeled ammonium nitrate with $^{15}N$ was supplied to the tobacco plant and compared with each other in the incorporation of nitrogen into the alkaloid. Although the incorporation of ammonium-nitrogen was more rapid than that of nitrate-nitrogen, there was no difference between them for 3 days after absorption.

## Effect of nutrient deficiencies

Alkaloid content was decreased with deficiencies of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron and boron. In the nitrogen deficient plant, synthesis of pyridine ring of nicotine was disturbed. This was obtained from the result that the incorporation of $^{14}C$-ornithine which is a precursor of pyrrolidine ring of nicotine into the nicotine was decreased with the nitrogen deficiency, but the one was recovered by the addition of nicotinic acid which is a precursor of pyridine ring of nicotine.

**Table 5. Incorporation ratios of DL-ornithine-2-$^{14}C$ into nicotine in the detached root of complete and nutrient deficient tobacco plants incubating with or without addition of nicotinic acid**

| | Without nicotinic acid | With nicotinic acid |
|---|---|---|
| Complete | 4.9 | 6.3 |
| –N | 2.0 | 6.1 |
| –P | 2.1 | 4.4 |
| –K | 1.8 | 2.8 |

(Ratios in percentage of radioactivity of nicotine to that of precursor fed)

**Table 4. Incorporation of ammonium and nitrate nitrogen absorbed from reciprocal $^{15}N$-labeled ammonium nitrate into alkaloid**

| | Nitrogen source | | | | | |
|---|---|---|---|---|---|---|
| | $NH_4{}^{15}NO_3$ | | | $^{15}NH_4NO_3$ | | |
| Hours after $^{15}N$-labeled-N supply | 5 | 24 | 72 | 5 | 24 | 72 |
| $^{15}N$-Total-N (mg/plant) A | 6.66 | 7.61 | 9.11 | 9.74 | 9.63 | 10.27 |
| $^{15}N$-Alkaloid-N (mg/plant) B | 0.021 | 0.106 | 0.480 | 0.120 | 0.266 | 0.544 |
| 100×B/A | 0.3 | 1.4 | 5.3 | 1.2 | 2.8 | 5.1 |

In the potassium and sulfur deficient plants, route from putrescine to pyrrolidine ring would be inhibited because the putrescine was accumulated in these plants. Decreases of alkaloid by nutrient deficiencies were not due to the degradation of alkaloid in the plant, but the inhibition of the synthesis. Demethylation of nicotine was accelerated with nitrogen and sulfur deficiencies.

## Effect of topping

Alkaloid content is increased with the topping which is commonly carried out in commercial cultivation. The reasons are as follows; translocation of nitrogen from leaves to the root and the activity of alkaloid synthesis are increased and degradation of alkaloid in the plant was inhibited by topping. The later the plant was topped, and the higher the topping position on the stalk was, the lower the alkaloid content was. These phenomena could be enlightened by the difference on the translocation of nitrogen in the plant and the accumulation period of alkaloid. In Japan, later topping and topping at the higher position on the stalk were commonly utilized in order to decrease the alkaloid content.

Usually, topping has been carried out at the flowering stage. But if the plant was topped at younger stages, alkaloid also increased. Increase of alkaloid synthesis is not a specific phenomenon during the ripening period but the ones accompanying topping.

## Differences of alkaloid content among tobacco varieties

In Japan, various types of tobacco have been cultivated. Alkaloid contents of domestic varieties which are air-cured type tobacco are lower than that of flue-cured ones. It was demonstrated, however, that there were no differences on the activity in the alkaloid synthesis among the varieties. This was obtained by the fact that there was little difference between the content and total amount of alkaloid in the air-cured type tobacco and those of flue-cured one when they were cultivated at the same condition.

The difference in the alkaloid content of the commercial tobacco would be attributed to the difference in the cultural practices, especially the period from topping to harvest of leaves.

In the air-cured tobacco, the ripening period is usually shorter than the flue-cured one; that is, the period of the accumulation of alkaloid is shorter in the air-cured type tobacco than in the flue-cured one. Additionally, lower alkaloid content in the air-cured type tobacco was also ascribed to the fact that the alkaloid was partially lost during air-curing.

## References


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