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# Relationship of Growth Characteristics and Leaf Surface Lipid of Tobacco

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# 잎담배의 엽면수지량과 생육형질과의 관계

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# 초 록

향끽미종잎담배인 소향의 시비량을 달리하여 엽면수지량과 생육형질과의 관계를 조사한 바, 주당 및 단위엽면적당의 엽면수지량은 질소수준간에 유의차를 보여  $3 \log / 10a$  수준에서 많았고 인산 및 가리의 영향은 유의성이 인정되지 않았다. 주당엽면적수지량은 초장, 최대엽장과 폭, 주당건엽중 및 생엽중과 평균엽면적과는 각각 정(+), 엽후와는 부(-)의 상관이 나타났다. 단위엽면적당 엽면수지량은 단위엽면적중 및 건물율과는 정(+), 평균엽면적과는 동일 질소수준에서 부(--)의 상관이 나타났다.

#### ABSTRACT

This experiment was conducted to investigate effects of fertilizers on leaf surface lipid (LSL), and relationship between growth characteristics and LSL of aromatic tobacco, cv. Sohyang, grown in pots with 3 rates of N, P and K.

With increasing the amount of applied nitrogen from 3 to 6kg/10a, the contents of LSL per plant and per leaf area were decreased. For maximum LSL per plant and per leaf area nitrogen of 3kg/10a was required There were little affected by P and k.

Leaf weight per plant and mean leaf area were correlated significantly and positively with LSL per plant, but thickness of largest leaf negatively. Specific leaf weight and leaf dry weight/leaf fresh weight ratio were correlated significantly and positively with LSL per leaf area, but mean leaf area negatively.

## INTRODUCTION

The leaves of tobacco are covered with a gummy layer, some of which seems to originate as an exudate from the leaf hair trichomes (12). The cuticle, which is the outermost layer of the leaf, is essentially cutin impregnated with wax. Cutin consists of cross -esterified polymerized hydroxy fatty acid, and the wax is mainly a mixture of hydro-carbons, alcohols, aldehydes, wax esters, and fatty acid (10, 11). In 1962 a new diterpene, 4, 8, 13-duvatriene-1, 3-diol was isolated from a hexane extract of aged tobacco (16) and more recently it was shown that the diterpene is a component of the cuticular wax (2, 12).

Various observations suggest that precursor of the characteristic aroma of tobacco may be derived from the exudate. For instance, the smoking aroma was drastically reduced by washing the tobacco leaf surface with chloroform and was almost completely recovered by addition of the washing onto the washed leaves (7). Another information shows that high quality leaves trend to be gummier than low quality leaves (4). Leaf surface lipids are readily extracted, without much contamination from internal lipids, by washing leaves in chloroform for 10 to 30 sec. (14), and with this technique 98% of the duvatrienediol was extracted from tobacco leaves (2).

Recently, a few papers (2, 5) were desc ribed that changes in total leaf surface lipid and duvatrienediol during tobacco growth, senescence and curing process and their alternation by soil condition and amounts of nitrogen applied on the tobacco field.

In this paper we describe the effect of fertilizer on leaf surface lipid and relationship of growth characteristics to leaf surface lipid of aromatic tobacco.

#### Materials and Method

An aromatic tobacco, Nicotiana tabacum

"Sohyang", was grown in a green-house of Eumseong Experiment Station, Korea Ginseng and Tobacco Research Institute. Nitrogen was applied at the rate of 0, 3, and 6 kg/10a, phosphate; 0, 9, 18, and potassium; 0, 18, 36. Experimental design was employed fractional factorial experimental design. Six plants per pot were transplanted on 16 April 1982. Pot spacing was 90cm × 45cm. Samples were taken at 55 days (flowering time) after transplanting. Whole leaves of 6 plants were used for each sampling.

Extraction and Determination of Leaf Surface Lipid. Leaf surface lipids were extracted using a slightly modified procedure after ref. (2, 5). Fresh tobacco leaves of each samples were weighed, leaf area measured, and dipped into 800ml chloroform for 30 sec. The extracts were filtered through Whatman 1 PS filter paper and were evaporated on a rotary evaporator at 40°C and weighed (Fig. 1).

Tobacco leaf (ca. 30 g)

Use weighed and area measured

Dipped into 800ml chloroform for 30 sec.

Filtered through Whatman 1PS filter paper

Evaporated on a rotary evaporator at 40°C

Weighed

Fig. 1. Extraction and determination of leaf surface lipid.

Residue

#### Results and Discussion

Growth Characteristics. Difference to the rate of nitrogen were significant for most growth characteristics (Table 1). Most growth characteristics were increased by increasing the rate of nitrogen fertilizer. Differences due to the rate of phosphate were significant for plant height and leaf dry weight/leaf fresh weight ratio. But differences due to the rate of potassium were insignificant. Jeong et al(3) reported that there were significant differences in growth characteristics due to nitrogen level but were insignificant differences with phosphate and potassium in flue-cured tobacco. The findings reported herein for Shoyang cultivar are in agreement with the flue-cured data

of Raper and McCants (15) and Kittrell et al (8).

Leaf Surface Lipid. In the leaf surface lipid contents per plant and per leaf area differences due to the rate of nitrogen were significant, but in the leaf surface lipid contents per fresh and dry weight of leaf, differences due to the rates and kinds of fertilizers were insignificant (Table 2). Leaf surface lipid contents per plant per leaf area were greater at the 3 kg than 6 kg/10a of nitrogen fertilizer rate. Nitrogen of 3 kg/10a was the conventional amount for the aromatic tobacco, and was known to produce the best quality leaves. The leaf surface lipid content of fresh leaves was 1.4-1.8mg/g. fr. wt., and of dried leaves 8.2-9.5mg/g. dry wt. (Table 2).

Table 1. Growth characteristics depending upon fertilizer levels at flowering time

Treatm	nent	Plant height	Length	Largest le Width	af Thickness	Mean leaf area	Leaf dry weight per plant	S.L.W*	L. D. W/ L. F. W ratio**
	kg/10a	cm	cam	cm	mm	cm²	g	g/1000cm <sup>2</sup>	%
Nitrogen	0	16.7	9.6	4.3	0.42	31.3	6.0	2.48	17.6
	3	28.5	12.0	6.1	0.37	52.5	11.0	2.74	18.7
	6	27.1	<b>13.</b> 1	6.2	0.37	58.6	9.9	2.21	17.0
Phosphate	0	22.0	12.0	5.6	0.38	49.0	8.6	2.31	16.3
	9	25.7	11.8	5.8	0.38	<b>50.</b> 9	9.6	2.48	18.1
	18	24.6	10. 9	5.2	0.39	42.5	8.7	2.64	18.9
Potassium	0	24.3	11.5	5.6	0.37	47.6	9.3	2.48	18.0
	18	24. 2	11.5	5.5	0.39	46.0	8.8	2.51	18.2
	36	23.8	11.7	5.5	0.39	48.8	8.8	2.44	17.2
LSD.05		2.7	2.5	1.4	0.04	11.4	1.7	0.38	1.8
LSD. 01		3. 9	NS	NS	NS	16.6	2.5	NS	2.6

<sup>\* ;</sup> Specific leaf weight

NS; Not significant at the 5% level of probability.

<sup>\*\* :</sup>Leaf dry weight/Leaf fresh weight.

LSD: Least significant difference.

Table 2. Leaf surface lipid depending upon fertilizer levels at flowering time

Treatment		per plant	per leaf fresh weight	per leaf dry weight	per leaf area	
	kg/10a	mg	mg/g	mg/g	$mg/1000cm^2$	
Nitrogen	0	8.1	1 .45	8. 2	26.5	
	3	17.4	1.80	9. 5	<b>34.</b> 0	
	6	<b>15.</b> 1	1.58	9.2	26.9	
Phosphate	0	12.4	1.42	8.7	26.6	
	9	14.5	1.64	9.0	29. 1	
	18	13.6	1.76	9.3	31.7	
Potassium	0	13.3	1.58	8.7	28. 9	
	18	13.8	1.70	9.3	30.4	
	36	13.4	1 .55	8.9	28.1	
LSD. 05		2.2	NS	NS	5.4	
LSD. 01		3.2			NS	

LSD; Least significant difference.

NS; Not significant at the 5% level of probability.

Chang and Grunwald (20) reported that the total surface wax content of fresh leaves was 1.2-1.4mg/g. fr. wt., and of air-dried leaves 7.3-8.0mg/g. dry wt. for Burley tobacco grown in green-house. Increased amount of applied nitrogen (6kg/10a) decreased contents of leaf surface lipid per plant and per leaf area (Table 2). Gamou and Kawashima (5) reported that increased amount of applied nitrogen decreased contents of leaf surface lipid and duvatrienediol, and also leaf quality for flue-cured tobacco.

Leaf surface lipid content per leaf area was 26.5-34.0 mg/1000 cm (Table 2). Gamou and Kawashima(5) reported that leaf surface lipid content was 112.7-167.0 mg/1000 cm leaf area for 25 th leaves after cotyledon at 100 days after transplanting of field grown flue-cured tobacco. In comparision with the tobacco leaves of Gamou and Kawashima(5), the leaves used in the present experiment contained smaller amounts of leaf surface lipid. The difference may be attributed to difference in cultivation and sampling conditions, where Gamou and Kawas-

hima used upper leaves grown in field. The leaves used in the present experiment were whole leaves (from bottom to top leaves), and were grown in green-house. Tobaccowas cultivated in our green-house and it was found that the amount of leaf surface lipid in these tobacco was about half compared with that of field grown tobacco. This finding is in agreement with the flue-cured tobacco data of Gamou and Kawashima(5).Chang and Grunwald (2) and Gamou and Kawashima (5) reported that comparing leaves on different stalk position, upper leaves always contained more leaf surface lipid at every stage. Bentley and Wolf(1) that the number of hairs per leaf of plants grown under glass (green-house) is strikingly less than that of plants of the same varieties grown in the field.

Relationship between Growth Characteristics and Leaf Surface Lipid. Plant height, largest leaf length and width, leaf dry weight and fresh weight per plant, and mean leaf area were correlated significantly and positively with leaf surface lipid per plant (Tab-

le 3). But thickness of largest leaf was correlated negatively with leaf surface lipid per plant.

Leaf dry weight/leaf fresh weight ratio and specific leaf area (SLW) were correlated significantly and positively with leaf surface lipid per leaf area, and the remaining growth characteristics were not corr elated significantly with leaf surface per leaf area (Table 3). Within the same nitrogen level, largest leaf length at the rate of 3kg/10a, and leaf fresh weight perplant at the rate of 0-3kg/10a and mean leaf area at the rate of 0-6kg/10a were correlated significantly and negatively with surface lipid per leaf area, respectively. In contrast, leaf dry weight/leaf fresh weight ratio and SLW at the rate of 0-6kg/10a were correlated positively with leaf surface lipid per leaf area, respectively. In tobacco plants, observers reported that plant height (6), largest leaf length and width (6, 9), and leaf area (17) were correlated positively with yield, and that thickness of leaf (9), and leaf dry weight/leaf fresh weight ratio (17) negatively. Miyazaki (13) reported that yield was correlated negatively with quality. In this research, we found that growth characteristics which have positive relationship with yield were correlated positively with leaf surface lipid per plant, and ones which have negative relationship with yield were correlated positively with leaf surface lipid per leaf area.

We conclude that nitrogen is the most critical element in determining the content of leaf surface lipid in aromatic tobacco, and that leaf dry weight/leaf fresh weight ratio, SLW, and mean leaf area within the same nitrogen level may be an acceptable selection criterion for quality of aromatic tobacco.

Table 3. Correlation coefficients between growth characteristics and leaf surface lipid

Growth characteristics	LSL per plant	LSL per unit leaf	LSL per leaf area Nitrogen level (kg/10a)		
		area	0	3	6
Plant height	0. 8964**	0.3471	0.0789	-0.0495	0.2612
Largest leaf length	0.6528**	-0.0573	-0.5292	-0.7010*	0.5601
Largest leaf width	0.8040**	-0.0756	-0.5870	-0.5919	0.3110
Largest leaf thickness	- 0.7141**	0.3149	-0.0181	0.1252	0.1241
Leaf dry weight per plant	0.8820**	0.2641	-0.3307	-0.0901	-0.0721
Leaf fresh weight per plant	0.7663**	0.0050	-0.6686*	-0.6039*	-0.2595
Mean leaf area	0.7158**	-0.2301	-0.9119**	-0.7521*	-0.7601*
L. D. W. /L. F. W. ratio	0.3270	0.6623**	0.7816**	0.8310**	0.2526
S. L. W.	0.1262	0.7225**	0.7722*	0.7811*	0.5348
Number of treatments	27	27	9	9	9

<sup>\*, \*\*:</sup> Significant at P<0.05 and P<0.01 levels, respectively.

LSL: Leaf surface lipid.

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