# たばこ(Nicotiana tabacum L.)の葉脈形成に関する遺伝学的研究


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# Genetical Studies on Vein Formation in *Nicotiana tabacum* L.

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## Summary

Two groups of lines, one irradiated by 30 KR X-rayed ($M_3$) and the other control ($C_3$), of the flue-cured tobacco plant (*Nicotiana tabacum* L.) were used in the experiment. Forty-four lines randomly selected from each of the $C_3$ and $M_3$ populations were compared. It was found that leaf vein number was more variable among $M_3$ lines than $C_3$ lines. By selection for higher or lower number of leaf veins in the $M_3$ and $M_4$ populations, an occurrence of some genic mutations due probably to X-ray irradiation for the formation of leaf veins was ascertained, though genetic variation was found only in decreasing the vein number.

An inquiry into genetic correlation between the number of leaf veins and some other quantitative characters in the $M_4$ and $M_5$ lines led us to conclude that genes responsible for vein number are more or less related to leaf size or shape genes, though the lower the leaf position on a stalk, the higher the degree of genetic correlation. Genes for increasing number of leaf veins made leaves longer and narrower. The number of leaf veins in leaves on the lower positions of a stalk had genetically closer relation with either leaf length, leaf shape or plant height.

Some discussions about the genetic control of organ formation in a higher plant were given.

## Introduction

A genetical study on plant organ formation or on the differentiation and development of plant organs is of interest and may be a very important subject because of its close relationship with problems for plant breeding.

Sakai and Shimamoto[6] reported that there were two ways of genetical approach in studying the differentiation and development of organs of higher plants : (1) by inquiring into the effect of mutation of major genes concerned with development, or (2) by quantitative analysis of developmental relations among different characters normal in appearance but variable in quantity. They also mentioned that there would be something like a key- or switch-gene responsible for normal organ formation because of occurrence of abnormal

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development of a plant organ by a single mutation, and that modifiers having individually minor effects operate coordinately in the development of plant parts and are probably responsible for phenotypic variation.

The present experiment was conducted to ascertain if such a fundamental plant organ as leaf veins including xylem and phloem could be genetically changed by inducing genetic mutations and to pursue the possibility of breeding a new type of tobacco plant having a very large or very small number of veins on each leaf through successive selections.

## Materials and Methods

Materials used in this experiment consisted of two populations derived from the flue-cured tobacco (*Nicotiana tabacum* L.) variety 'Hicks'. One population was a control or not irradiated, being designated as C, while the other one was X-rayed by 30 KR, being called M. The two populations were propagated by one-plant-one-offspring breeding method in each of the succeeding years. This method was adopted in order to free the population as much as possible from natural selection due to some differences in the indvidual contribution to the next generation. The 200 plants randomly selected from each of two populations ($C_1$ and $M_1$) and sown in nursery bed were planted and each plant was allowed to produce only one progeny ($C_2$ and $M_2$) based on the propagation procedure as above mentioned.

Sampling of the tobacco leaves for the investigation was conducted two weeks after the first flowering of the plant. Five leaves on the middle part of a stalk were removed from the $C_2$ and $M_2$ plants in order to count the number of veins per leaf. The veins investigated in this experiment concerned with the principal lateral veins of a leaf excepting the midrib and minor veins distributed near the base or tip of a leaf (see Fig. 1).

Both $C_3$ and $M_3$ populations were composed of 44 lines which were grown from plants randomly selected from each of the $C_2$ and $M_2$ populations. From among the 44 lines of $M_3$ population six lines were selected for higher and five for lower number of veins per leaf.

All materials were planted in randomized plots with two replications. Number of plants sampled per plot was 10 in either $C_3$ or $M_3$ generation and 8 in $C_4$ or $M_4$ generation. Tobacco leaves sampled for investigation were four largest ones growing on the middle of a stalk in the $C_3$, $M_3$ and $M_4$ populations and all leaves on a stalk in $M_5$ population. Selection for number of veins per leaf from the $M_4$ lines was practiced on the basis of a combined value of a line mean

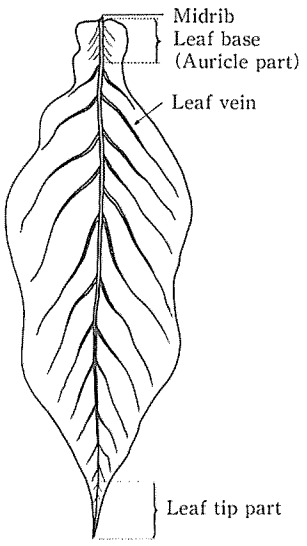


Fig. 1 Schematic figure of tobacco leaf veins
The leaf base and tip parts were not included in the counting.

and a plant performance. The leaves sampled from a whole plant in the $M_5$ line were classified into three groups due to their position on a stalk, i. e. the upper (three leaves on the highest part of a stalk except for the smallest one on the top), the middle (four leaves on the middle part of a stalk or those below the upper group) and the lower (ca. four leaves on the lowest part of a stalk or those below the middle group).

The cultural practices of progeny plants were the same as those conventionally adopted for flue-cured tobacco production in Japan. One plot was composed of a single row of 15 competitive plants spaced 40 cm in the row with a spacing 90 cm between rows.

The realized heritabiltiy[2] was estimated from the formula $h^2=G/i$, where i= the selection differential or the deviation of the mean of the selected group from that of the original popultion, while G=the genetic gain or the deviation of the selected progeny group from the population mean. The 95% confidence interval of heritability due to the parent-offspring regression coefficient was obtained by the following formula[3] :

$$H^2_{RS} \pm t_{.05}\sqrt{(S_o - S^2_{OP}/S_P)/(n-2)S_P}$$

where $S_o$ = the sum of squares of the offspring

$S_P$ = the sum of squares of the parents

$S_{OP}$ = the sum of products between the parents and the offsprings

n = number of pairs

## Results

Fig. 2 shows the frequency distribution of number of veins per leaf in populations of $C_3$ and $M_3$ lines and the analyses of variance for the vein number are presented in Table 1. As shown in Fig. 2 the variation of number of veins per leaf among $M_3$ lines is obviously wider than that of $C_3$ population though no significant differences between the mean values was detected. Significant differences among lines of the vein number were found in both $C_3$ and $M_3$ populations as shown in the analyses of variance(Table 1.). The estimates of genetic variance or the variance component due to lines and the environmental variance or the variance among competitive plants within a plot were obtained from the analyses of variance as shown in Table 1. The estimate of the genetic variance thus obtained is 2. 33 in $M_3$ population, that is 1. 44 times larger than that of $C_3$ population, while the environmental vari-

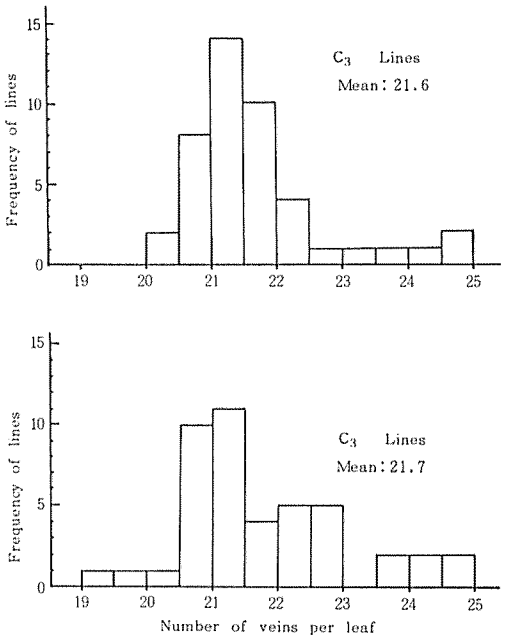


Fig. 2 Frequency distribution of lines for the number of veins per leaf in $C_3$ and $M_3$ populations

Table 1 Analyses of variance of number of veins per leaf in control($C_3$) and irradiated($M_3$) populations of the tobacco variety 'Hicks'

| Source of variation | $C_3$ | | $M_3$ | | Expectation of M.S. |
|---|---|---|---|---|---|
| | d.f. | M.S. | d.f. | M.S. | |
| Replications | 1 | 150.45 | 1 | 11.52 | $\sigma_e^2+k_2\sigma_L^2+k_3\sigma_R^2$ |
| Lines in Rep. | 86 | 61.71** | 86 | 87.86** | $\sigma_e^2+k_1\sigma_L^2$ |
| Plants in Plot | 3166 | 1.71 | 3162 | 1.86 | $\sigma_e^2$ |

$k_1$ : Number of plants per plot, 36.97 in $C_3$ and 36.93 in $M_3$ respectively
** : Significant at 1% level

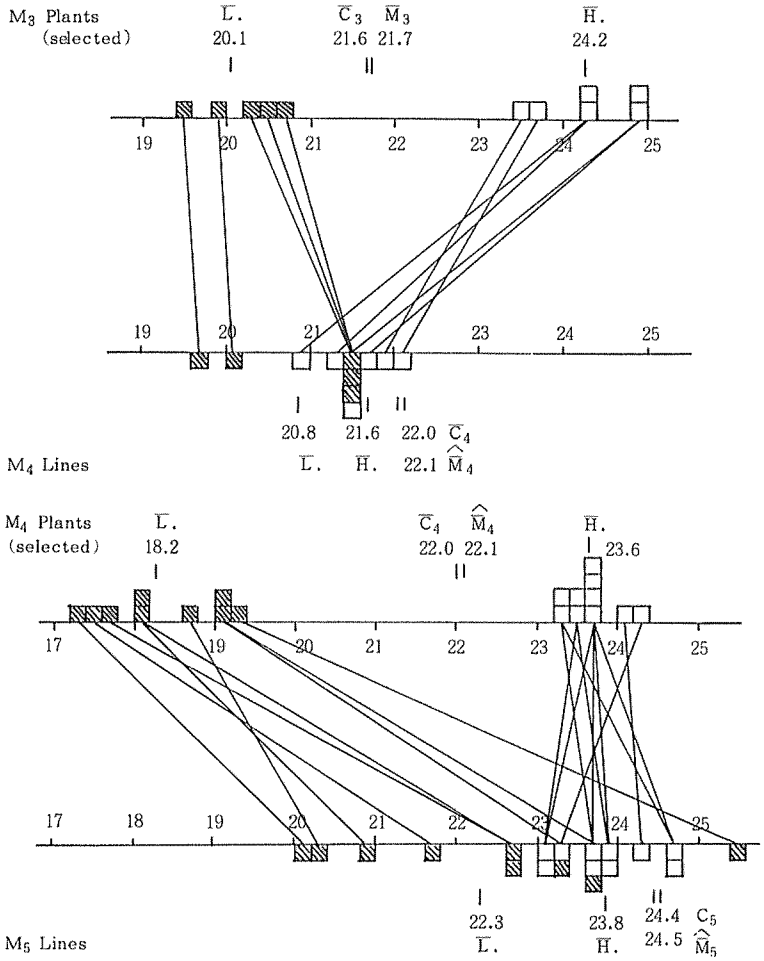


Fig. 3 Processes of selection for and against the number of veins per leaf in the $M_3$ and $M_4$ populations
$\bar{C}_4$ : Mean value of five $C_4$ lines randomly selected from $C_3$ population
$C_5$ : One $C_5$ line bred from the mixed seeds of five $C_4$ lines
$\hat{M}_4$, $\hat{M}_5$ : Mean values of $M_4$ or $M_5$ populations, estimated from the equations, $\bar{M}_3+\bar{C}_4-\bar{C}_3$ or $\hat{M}_4+C_5-\bar{C}_4$

ances in both populations are almost the same as shown in Table 1.

These facts suggest that some gene mutation concerning the leaf vein formation might have occurred in the $M_3$ population due to X-ray irradiation, and also that the original tobacco variety 'Hicks' is considered to have in itself some levels of genetic variation for the leaf vein formation.

Details of the result of selection for and against the numer of leaf veins are shown in both Table 2 and Fig. 3, from which we understand that the selection was in general effective for smaller number of veins in either $M_3$ or $M_4$ population. The realized heritabilities by the selection for the smaller number of veins obtained from the formula, $h^2=G/i$, were 0.800 from the selection in $M_3$ population, and 0.587 from that in $M_4$. The estimates and their 95% confidence intervals of heritability obtained from the parent-offspring regression and the formula above mentioned[3] were $0.191 \pm 0.201$ in the case of selection in $M_3$ and $0.315 \pm 0.211$ in the case of selection in $M_4$. These results show that the genetic variability of the number of leaf veins due to X-ray irradiation might have occurred only for lesser number of veins.

Some genetical parameters for the number of leaf veins and some other quantitative

Table 2 Number of veins per leaf after successive selection for and against their numbers in $M_3$ and $M_4$ lines derived from the irradiated population of the tobacco variety 'Hicks'

| $M_3$ | | $M_4$ | | | $M_5$ | |
|---|---|---|---|---|---|---|
| Line No. | Mean | Mean | Within L. Variance | Plant Value (selected) | Line No. | Mean |
| L. 93 | 19.88 | 20.11 | 1.684 | 18.00 | S. 1 | 22.64 |
| | | | | 17.75 | S. 2 | 22.65 |
| | | | | 17.50 | S. 3 | 21.78 |
| | | | | 24.25 | S. 10 | 23.31 |
| L. 97 | 20.52 | 21.51 | 0.731 | (discard) | | |
| L. 101 | 20.62 | 21.44 | 1.877 | 19.00 | S. 4 | 23.38 |
| | | | | 19.25 | S. 5 | 25.41 |
| | | | | 24.00 | S. 11 | 24.21 |
| | | | | 23.75 | S. 12 | 23.71 |
| L. 121 | 19.41 | 19.75 | 0.826 | 18.00 | S. 6 | 20.98 |
| | | | | 18.75 | S. 7 | 20.34 |
| | | | | 17.25 | S. 8 | 20.01 |
| L.132 | 20.24 | 21.56 | 0.752 | (discard) | | |
| L. 163 | 23.57 | 21.89 | 0.572 | 23.50 | S. 13 | 23.06 |
| L. 165 | 23.64 | 22.11 | 1.432 | 23.25 | S. 14 | 23.67 |
| | | | | 23.75 | S. 15 | 24.64 |
| L. 167 | 24.94 | 21.78 | 0.917 | (discard) | | |
| L. 171 | 24.83 | 21.55 | 1.213 | 23.25 | S. 16 | 24.73 |
| | | | | 23.75 | S. 17 | 23.88 |
| L. 181 | 24.26 | 21.28 | 0.774 | (dicard) | | |
| L. 190 | 24.21 | 20.97 | 1.189 | 19.00 | S. 9 | 23.64 |
| | | | | 23.75 | S. 18 | 23.13 |
| | | | | 23.50 | S. 19 | 23.99 |

characters estimated in $M_4$, and also $M_5$, where leaves on upper, middle and lower position on a stalk were separated, are shown in Table 3. All of the characters including the number of leaf veins showed wide among-line variation in both $M_4$ and $M_5$ populations. The variation was always statistically highly significant for all characters investigated.

Heritabilities estimated from the analyses of variance were in general rather low for all characters except for number of leaf veins and leaf shape index (See Table 3). The heritability values for the leaf characters in $M_5$ population tended to increase as the position of leaves came down on a stalk except for the leaf shape index which had the largest value on the highest position on a stalk.

Table 4 shows the genetic and environmental correlations between the number of leaf

Table 3 Population means, range of line means within each group, and mean squares among the lines and among plants within each line
Estimates of heritability ($h^2$) from the analysis of variance of the vein number per leaf and of some other quantitative characters in $M_4$ and $M_5$ lines are also described.

| Character | | | Population Mean | Range | Mean Squares Lines | Mean Squares Plants/L. | $h^2$ |
|---|---|---|---|---|---|---|---|
| Number of veins per leaf | $M_4$ | | 21.3 | 19.8 – 22.1 | 6.860** | 1.089 | 0.398 |
| | $M_5$ | Up.+ | 21.5 | 18.9 – 22.8 | 13.594** | 2.280 | 0.382 |
| | | M | 23.1 | 20.0 – 25.4 | 19.126** | 2.388 | 0.467 |
| | | Lo. | 22.3 | 18.6 – 24.6 | 22.906** | 2.773 | 0.476 |
| Leaf Length (cm) | $M_4$ | | 66.0 | 62.2 – 67.9 | 54.255** | 14.073 | 0.263 |
| | $M_5$ | Up. | 58.3 | 54.0 – 64.1 | 139.792** | 46.029 | 0.202 |
| | | M. | 65.4 | 59.9 – 69.4 | 91.133** | 30.455 | 0.199 |
| | | Lo. | 62.6 | 52.7 – 67.5 | 134.071** | 28.988 | 0.311 |
| Leaf Width (cm) | $M_4$ | | 25.3 | 23.9 – 27.0 | 14.128** | 4.789 | 0.196 |
| | $M_5$ | Up. | 17.6 | 15.7 – 19.0 | 19.392** | 7.500 | 0.165 |
| | | M. | 21.4 | 18.9 – 23.2 | 17.565** | 4.796 | 0.249 |
| | | Lo. | 22.3 | 19.8 – 24.3 | 20.470** | 5.013 | 0.278 |
| Leaf Shape log(L./W.) M.S. : x 1000 | $M_4$ | | 0.412 | 0.364–0.427 | 6.149** | 1.069 | 0.373 |
| | $M_5$ | Up. | 0.523 | 0.468–0.587 | 8.379** | 1.365 | 0.391 |
| | | M. | 0.488 | 0.447–0.542 | 5.562** | 1.074 | 0.343 |
| | | Lo. | 0.444 | 0.405–0.489 | 5.258** | 1.287 | 0.278 |
| Leaf Area log(L. x W.) M.S. : x 1000 | $M_4$ | | 3.210 | 3.171–3.246 | 8.316** | 3.254 | 0.163 |
| | $M_5$ | Up. | 2.999 | 2.945–3.086 | 33.711** | 14.501 | 0.142 |
| | | M. | 3.141 | 3.076–3.197 | 17.932** | 5.928 | 0.202 |
| | | Lo. | 3.142 | 3.014–3.202 | 27.220** | 5.789 | 0.316 |
| Number of Leaves per Plant | $M_4$ | | 18.4 | 17.8 – 19.3 | 5.357** | 2.082 | 0.164 |
| | $M_5$ | | 16.1 | 13.1 – 19.0 | 24.420** | 5.203 | 0.316 |
| Plant Height (cm) | $M_5$ | | 99.2 | 83.6 –109.1 | 528.514** | 168.109 | 0.211 |
| Stem Diameter (cm) | $M_5$ | | 2.58 | 2.37– 2.76 | 0.108** | 0.056 | 0.106 |

+ : Up., M., Lo. ; Upper, middle and lower groups of leaves on a stem
Degrees of freedom in the analyses of variance : 20($M_4$) and 36($M_5$) for among lines, 175 ($M_4$), 260(upper and middle groups in $M_5$) and 241(lower group in $M_5$) for among plants/line.
** : significant at 1% level

veins and other quantitative characters in $M_4$ and $M_5$ lines. Leaves sampled from the $M_5$ lines were classified into three leaf-height groups as mentioned above. In the $M_4$ lines the number of leaf veins was positively correlated with shape and length of leaves. Leaf area and number of leaves on a stalk was found to be little correlated with the number of leaf veins. Environmental correlations between the number of leaf veins and other characters were in general small except for leaf length.

Genetic correlations between the number of leaf veins and other characters in $M_5$ population were variable among the leaf groups or due to the leaf position on a stalk. Of three groups in $M_5$ lines, genetic correlations between the number of leaf veins and other characters except for leaf width were in general higher in the lower leaf group than those of other two groups. This fact may apparently tell that plants having leaves with larger number of veins may coincidently have some genetical ability to make the leaves longer and also narrower in shape. Might it not be taken to suggest that plant organs in early stages of plant development could be more intensively governed by relevant genes than later stages? The reason is because the genetic correlation between the vein number of leaves developed on the lower part of the stem and some growth characters such as plant height, leaf length, leaf size and leaf number are particularly high in comparison with others.

Variation in the number of veins per leaf collected from different positions on the stalk in a control and four $M_5$ lines are shown in Fig. 4. Of the four $M_5$ lines, two(S-7, 8) produced leaves with smaller number of veins, while the other two(S-15,16) larger number. Leaves with the largest number of veins were generally found on the middle part of a stalk or at the 6th to 7th downward from the top, though in the two $M_5$ lines with smaller number of veins the patten of change was less apparent than the other two. Similar positional change in the leaf length in the same five lines are shown in Fig. 5. It is of interst to find in the same figure that the leaf length of all $M_5$ lines was likely to be more or less shorter

Table 4 Genetic and environmental correlations between number of veins per leaf and some other quantitative characters in $M_4$ and $M_5$ lines

| | Number of leaf veins | Leaf Character | | | | | Plant Height | Stem Diameter |
|---|---|---|---|---|---|---|---|---|
| | | Length | Width | Shape | Area | Number | | |
| $r_G$ | $M_4$ Middle four lvs | 0.305 | −0.238 | 0.398 | −0.066 | −0.060 | — | — |
| | $M_5$ Upper group | 0.218 | −0.249 | 0.539 | −0.075 | 0.040 | 0.312 | 0.346 |
| | $M_5$ Middle group | 0.410 | −0.124 | 0.481 | 0.129 | 0.201 | 0.526 | 0.300 |
| | $M_5$ Lower group | 0.741 | 0.099 | 0.686 | 0.498 | 0.381 | 0.738 | 0.399 |
| | $M_5$ Pooled* | 0.296 | −0.178 | 0.499 | 0.030 | — | — | — |
| $r_E$ | $M_4$ Middle four lvs | 0.447 | 0.102 | 0.229 | 0.263 | 0.090 | — | — |
| | $M_5$ Upper group | 0.339 | −0.088 | 0.058 | 0.284 | −0.040 | −0.019 | 0.147 |
| | $M_5$ Middle group | 0.603 | 0.170 | 0.383 | 0.409 | −0.147 | −0.006 | 0.147 |
| | $M_5$ Lower group | 0.570 | 0.295 | 0.190 | 0.480 | 0.104 | 0.228 | 0.208 |
| | $M_5$ Pooled* | 0.456 | 0.028 | 0.212 | 0.325 | — | — | — |

* : Upper and middle groups are pooled.
$r_G$, $r_E$ : genetic and environmental correlations

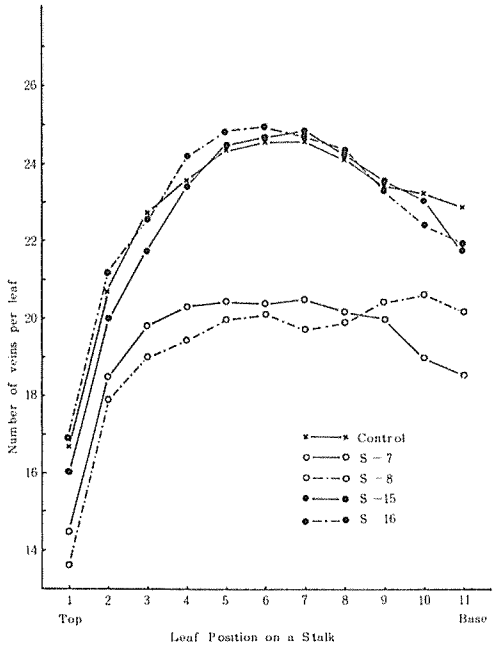


Fig. 4 Variation in the number of veins per leaf due to the position on a stalk in the control and four $M_5$ lines

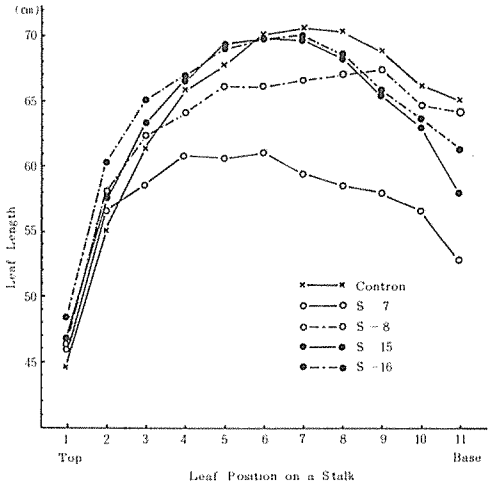


Fig. 5 Variation of leaf length due to the position on a stalk in the control and four $M_5$ lines

than the control except for a few leaves of the higher positions.

## Discussion

Venation of *N. tabacum* leaf is characterized by midvein, principal lateral veins and the coarse vascular network, in which xylem and the external and internal phloem are found. These xylem and phloem are concerned with mechanical support for a leaf as well as conduction and storage of food and water which are absolutely essential for preservation of life.

Accodingly, genetic study concerning the formation of such a fundamental organ as leaf veins should be a fascinating theme for us when we try to solve the problem of evolution of higer plants on the earth.

In fact, however, genetical study of the vein formation in a higher plant had little been conducted so far. Sakai and Shimamoto[7] investigated genetically a developmental problem of tobacco leaf veins through the developmental instability measured by bilateral asymmetry or intra-leaf variability of vein distribution. They concluded from their study that developmental instability in leaves varied depending upon the developmental stage; the variation in the instability or stability among varieties was found to be greatest at the middle stage of growth of a tobacco plant, which suggested that genes responsible for the leaf development might become more active at a certain growth period than other stages.

Crookston and Moss[1] reported that a large difference was present in leaf vein formation in C-4 and C-3 species of plants. They found that vascular bundle sheaths in the C-4 species were separated by two rows of mesophyll cells only, whereas veins in the C-3

species were separated by 13 rows, on the average, of mesophyll cells.

Hanson and Rasmusson[5] conducted a survey of leaf vein frequency in 210 varieties of barley (*Hordeum vulgare* L.) and found a great variation among varieties. From the selection experiment they estimated the mean value of realized heritability to be $0.20\pm0.04$ in five $F_2$ populations and $0.45\pm0.07$ in $F_3$ populations for the leaf vein frequency. They also found that the selection response from both the $F_2$ and $F_3$ generations was asymmetrical with more gain in selection for higher than for lower frequency. Leaf vein frequency was negatively associated with length, area and dry weight of leaves.

Genetical study on the midrib proportin in tobacco plants was conducted by Iyama[4]. His findings were that varietal differences of the ratio of fresh or dry weight of midrib (midvein) to total leaf weight or midrib proportion were highly significant and genotypic correlations between the midrib proportion based on the fresh weight and the leaf shape (W./L.) was $-0.582$ and that with leaf area 0.360. He estimated the values of heritability, 0.320 in $F_2$, 0.407 in $F_3$ bulk and 0.590 in $F_3$ progeny with the number of effective factors, 4.10 as $K_1$ and 4.77 as $K_2$.

Our experimental data show that gene mutation in formation of leaf veins had occurred toward decreasing the number of which veins was genetically related with leaf length, leaf shape and plant height with higher levels of correlation in the lower position of leaves on a stalk.

These genetical studies concerning leaf vein formation in higher plants as above mentioned have presented some evidences that the formation of leaf veins are controlled by genes and it is more or less correlated genetically with some other quantitative characters of a leaf. It will be an interesting work in future to investigate what role do genes play in the formation of such a basic organ as a leaf in plants.

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# たばこ(*Nicotiana tabacum* L.)の葉脈形成に関する遺伝学的研究

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昭和62年4月30日

## 摘　　要

黄色種タバコ品種，ヒックスに由来する2組の集団，30KRのX線照射区と対照区を本実験に供試した．$C_2$及び$M_2$集団のそれぞれから任意に抽出した44系統の$C_3$及び$M_3$集団についてその葉脈数を比較したところ，$M_3$系統間の変異は$C_3$集団に比べて大きくなっていた。$M_3$及び$M_4$集団から葉脈数についてくり返し選抜を行った．その結果，X線照射によって葉脈形成に関する遺伝子突然変異が生じていることが分かった．しかし，その遺伝的変異は葉脈数を少くする方向に対してであった．

$M_4$及び$M_5$系統における葉脈数と他のいくつかの量的形質との遺伝相関をみると，葉脈数を多くするような遺伝的能力は一方葉を長く，細くする傾向にあり，また，下位葉の葉脈数が，葉長，葉形及び草丈とより密接な遺伝的関係をもっていることが分かった．これらの関係は葉面積及び茎の直径においてもその傾向がみられた．一方，葉脈数と他の形質との環境相関は低かったが，葉長及び葉面積との間には正の相関がみられた．これらの結果をふまえて，高等植物の器容形成の遺伝的支配について2，3の論議を行った．