Haploid Plant Characteristics and Screening for T.M.V. Resistance from in Vitro Anther Culture of *Nicotiana tabacum* L.

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담배 葯培養에 의한 半數體植物의 特性과 T.M.V. 抵抗性 檢定

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ABSTRACT

Production of haploids in vitro anther culture of *Nicotiana tabacum* L. was oriented in a large number on a chemically defined culture medium. The haploids were screened for T.M.V. resistance and the segregating ratio in F_1 were in good agreement with the expected ratio.

INTRODUCTION

The potential benefits of haploid production to crop improvement were expressed by many researchers[6,9,13,14,21].

The induction of haploid plants through anther culture in tobacco was attempted and accomplished [9,14,21], but the induced number of haploid plants was quite low. To date, therefore, few workers have utilized anther-derived haploids in tobacco breeding program. Hence, our objective of this study is oriented toward production of large numbers of hapoids from specific genetic stocks and screening them for disease resistance, especially for tobacco mosaic virus (T.M.V.).

MATERIALS AND METHODS

Plant materials. Two varieties, 'S.C. 72' and 'Hicks', and their single cross F_1, 'S.C. 72' x 'Hicks', were subjected for this study. Floral buds used for the culture of anther-derived haploids were obtained from field and greenhouse-grown tobacco plants. The floral buds were collected when the corolla was visible just beyond the calyx, described as "Stage-2" by Nitsch and Nitsch[18].

Surface sterilization The floral buds excised from tabacco plants were immersed directly in 95% ethanol for two to three seconds and followed by calcium hypochlorite (v/v) solution for ten to 15 minutes. The disinfected materials were rinsed several times in autoclaved distilled water, then the anthers were excised from the surface sterilized floral buds and placed on the culture medium in standard 200ml erlenmeyer flask.

Anther culture. Stage of anther development determined through the cytological examination using acetocarmine squach method for a anther in each floral bud. Anther length in uninucleate stage is about 1.7 or 1.8 cm. Ten excised anthers per flask were placed on 40ml of medium. The medium for the anther culture in this study was D-medium of Nakamura et al. [19], which is the basal medium of Nakata and Kurihara[14] plus 3g/liter active carbon and without kinetin. The cultural chamber

was maintained at 25±1°C and was illuminated continuously at about 3,000 Lux from cool white fluorescence lamps located above the chamber. Air movement in the chamber was kept in minimal. Thus, drying of the anthers and/or medium was not a serious problem

Cytological evaluation. To determine the level of ploidy root tips of anther derived plants were examined using a modified venetian turpentine mounting medium outlined by Haunold[1]. Root tips were fixed in the Farmer's fluid and stained in Schall's staining solution.

Screening procedure of T.M.V.(Tobacco mosaic virus). Tobacco leaves showing systemic mosaic symptoms were excised from the stalk, then pressed out juic efrom them and diluted the juice to 10^{-3} with 0.1 M-phosphate buffer solution adjusted to pH 7.0. Haploid plants with eight to ten leaves were inoculated with diluted solution by rubbing method and identified its resistance to mosaic virus.

Resistant plant showed the typical necrotic symptom and the leisons were not proceeded.

Measurements. Plant height in cm, total leaf number,and leaf shape in leaf width/leaf length were measured for all haploid plants except the plant susceptible to T.M.V. at the flowering stage.

RESULTS AND DISCUSSION

Plantlets start to emerge from anthers cultured about 30 days after their culture on the medium defined chemically and continued to about 50 days. Total number of anthers cultured were 30, 35 and 95 for 'Hicks', 'S.C.72', and their single cross respectively. Number of anthers produced haploid at different dates were as shown in Table 1.

The percentages of anther inducing haploid plants from the attemped anthers were over 30% for each of plant materials(Table 1.) (Fig. 1). Mean number of haploid plantlets per anther in vitro anther culture were over six plants per anther for each plant materials (Table 2.). X^2-test for T.M.V. resistance in single cross F_1, 'S.C.72' x 'Hicks' suggested that the observed segregation ratio of T.M.V. resistance were in good agreement with 1 : 1 ratio, which is the theoretically expected one(Table 3.). The ranges of haploid plant characteristics

Table. 1. Number of anthers from which haploid plant is derived in vitro anther culture at different dates.

Varieties and single cross	Total no of anthers cultured	No. of anthers produced haploid at different dates						
		30 days	35 days	40 days	45 days	50 days	Total	Percent
Hicks	30	3	5	—	2	—	10	33.3
S.C.72	35	4	2	1	3	1	11	31.4
S.C.72×Hicks	95	7	5	6	9	2	29	30.5

Fig. 1. Haploid plantlets emerged from anthers on medium.

Table. 2. Mean number of haploid plantlets per anther in vitro anther culture

Varieties and single cross	No. of haploid plantlet per anther			
	1—5	6—10	11	Average
Hicks	5	2	3	6.7
S.C.72	5	4	2	6.5
S.C 72×Hicks	14	11	4	6 1.

Table 3. X^2-test for tobacco mosaic virus(T.M.V.) resistance in single cross F_1, 'S.C.72'×'Hicks'

Item	Resistance	Susceptible	Totals
Observed(O)	114	136	250
Expected(E)	125	125	250
$\frac{(O-E)^2}{E}$	0.968	0.968	*X^2=1.936

$X^2_{0.05(1)}=3.84$

Table. 4. Means and ranges of haploid plant characteristics at the flowering stage

Varieties and single cross	Plant height (cm)		Total leaf no.		Leaf shape index	
	Mean	Range	Mean	Range	Mean	Range
Hicks	71.4	47.0—104.0	19.4	17—24	0.36	0.27—0.43
S.C.72	63.6	45.5— 96.5	21.2	16—32	0.33	0.24—0.42
S.C.72×Hicks	54.1	30.0—100.5	17.6	13—33	0.31	0.21—0.45

measured showed that the haploid from single cross F_1 gave more deviation than those from either parents (Table 4.).

SUMMARY

This study was conducted to utilize the haploid method for the practical breeding of *Nicotiana tabacum* L. using the "D" medium of Nakamura. The results obtained were summarized as follows:

1) From the attempted anther culture, over 30% of anthers in the culture medium produced haploid plants and the average number of derived haploid plant per anther were over six plants.

2) Observed segregating ratio of T.M.V. resistance among haploid plants derived from the single cross F_1, were in good agreement with 1 : 1 ratio, that is, the theoretically expected one.

3) The ranges of haploid plant characteristics derived from the single cross, 'S.C.72' x 'Hicks' F_1, were deviated more than those of their parents, as we expected.

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摘　　要

담배에서 葯培養에 依한 半數體育種을 實用化하고저 中村等의"D"培地를 利用하여 實驗을 하였든바 그 結果를 要約하면 다음과 같다.

1. 培養葯中 平均 30% 以上이 半數體植物을 誘起하였으며 葯當 誘起된 植物個體數는 平均 6株以上이었다.

2. F_1으로부터 誘起된 半數體中 T.M.V. 抵抗性은 理論値와 一致하는 1 : 1의 比를 나타내었다.

3. 半數體植物의 生育調査에서는 豫期한바와 같이 모든 測定形質에 對하여 F_1의 半數體가 母本의 半數體보다 더큰 變異를 보였다.