Competition among Pollen Grains for Haploid Tobacco Plant Formation by Anther Culture

II. Analysis with Resistance to Tobacco Mosaic Virus(TMV) and Wildfire Diseases, Leaf Color, and Leafbase Shape Characters

KAZUO NAKATA and TSUNE KURIHARA
Hatano Tobacco Experiment Station, Hatano

Synopsis. The segregation obtained for several characters in haploid tobacco plants developed from pollen grains of the F_1 hybrids showed a good fit to the expected ratios. This indicates that no competition could be seen among pollen grains in the formation of haploid plants by anther culture. Anther culture technique is applicable to gene analysis.

Introduction

It is not well-known whether the heritable characters segregate with the expected ratios in the haploid tobacco plants derived from pollen grains. In connection with the previous paper (NAKATA 1969, 1971), the resistance to TMV and wildfire diseases, the leaf color, and the leafbase shape were chosen as the characters for experiments.

HOLMES(1938) and VALLEAU(1949) proved that *Nicotiana glutinosa* carries N factor for resistance to TMV. This type of resistance was first transferred from *N. glutinosa* to *N. tabacum* by HOLMES(1938). In addition, CLAYTON (1947) reported that the dominant factor from *N. longiflora* for immunity to wildfire diseases is simple. Resistance to each of these diseases shows completely dominant monogenic inheritance. The white character(due to chlorophyll deficiency) was chosen as the leaf color character. This character is governed by two double recessive genes(HENIKA 1932). It was found by CLAUSEN and CAMERON(1944), VAN DER VEEN (1957), and HUMPHREY *et al.* (1964) that the inheritance of the leaf shape is controlled by three pairs of genes. More recently, EGUCHI (1971) reported the same results as those of HUMPHREY *et al.*(1964) and postulated the genotypic models for twentyseven varieties of the flue-cured tobacco in which the genotypes for Bright Yellow 4(or Hicks 2) and Golden Cure are described as PtPtpdpdBrBr and ptptPdPdBrBr, respectively. It was also designated by

him that the genotype for Burley 21 is ptptPdPdBrBr and NFT 706 is ptptpdpdbrbr (personal communication).

This paper reports that these characters mentioned above segregate with the expected ratios in haploid tobacco plants growing in soil. Thus, evidences supporting the view that this technique is useful in gene analysis were enhanced. A preliminary report was published(NAKATA and KURIHARA 1971).

Materials and Methods

The five F_1 hybrids among cultivated varieties of *N. tabacum* L.; One-Sucker $\times$ Burley 21, Golden Cure $\times$ Burley 21, Bright Yellow 4 $\times$ Burley 21, Hicks 2 $\times$ Burley 21, and NFT 706 $\times$ Burley 21 were obtained in Hatano Tobacco Experiment Station in 1969. Anthers from these five F_1 hybrids were used for culture.

The composition of the medium used in this study was as follows in mg/litre. KNO_3 , 250.0; $Ca(NO_3)_2 \cdot 2H_2O$, 300.0; KH_2PO_4 , 100.0; $MgSO_4 \cdot 7H_2O$, 100.0; $MnSO_4 \cdot 4H_2O$, 10.0; $FeSO_4 \cdot 7H_2O$, 27.85; $Na_2 \cdot EDTA$, 37.35; IAA, 2.0; kinetin, 1.0; myo-inositol, 100.0; thiamine hydrochloride, 0.4; adenine sulphate, 10.0; sucrose, 30,000.0; agar, 6,000.0. In some cultures, 550.0 mg/litre of NH_4NO_3 was used instead of 300.0 mg/litre of $Ca(NO_3)_2 \cdot 2H_2O$.

Burley 21 is resistant to both TMV and wildfire diseases, and a chlorophyll deficient type (HEGESTAD *et al.* 1960). In contrast to Burley 21, One-Sucker, Golden Cure, Bright Yellow 4, Hicks 2 and NFT 606 are all sus-

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ceptible to TMV and wildfire diseases, and a green type.

The ordinary strain of TMV and PT-3 strain of *Pseudomonas tabaci* (WOLF & FOSTER) STEVENS were used for inoculation. Detached leaves were cut from each haploid plant growing in soil and inoculated with either TMV or *P. tabaci*. Inoculated leaves were kept on the filter paper moistened with distilled water in petri dische and allowed to incubate for 5 to 9 days at 20~25°C. Each treatment was replicated 2 to 3 times.

Identification of the leaf color character was done shortly after haploid plants had flowered. The leafbase shape index were observed and calculated on the largest leaf when it reached 20~25 cm in length. Width of the leaf was measured at the widest of lamina and length was measured from the origin on the stalk to the tip of the leaf. Also, a ratio of the petiole to the leaf length was measured.

In addition, segregation for the resistance to TMV and wildfire diseases, and the leaf color character of F_2 generation in the cross of Hicks 2 $\times$ Burley 21 was observed.

Results

Development of the whitish plantlets from pollen grains

Haploid tobacco plantlets appeared from anthers of each F_1 hybrid with equal facilities. The whitish plantlets rarely emerged from anthers regardless of cross combination. A few whitish plantlets turned green in short after they had been transplanted to soil, and grew to flowering stage. However, most of the whitish plantlets failed to grow in soil due to either poor development or lack of roots. The ratio of the whitish to the green plantlets was not constant on medium.

Segregation for resistance to TMV in haploid tobacco plants

Resistance to TMV is governed by a single dominant gene. Thus, a 1 resistant : 1 susceptible would be expected in haploid plants developed from pollen grains of each F_1 hybrid. As given in Table 1, segregation for resistance to TMV in those from the five F_1 hybrids was in satisfactory agreement with a 1 resistant : 1 susceptible ratio.

Segregation for resistance to wildfire disease in haploid tobacco plants

Table 1. Segregation for resistance to TMV in haploid tobacco plants emerged from anthers of the F_1 hybrids

Cross combination	Resistant	Susceptible	No. of plants	χ^2 (1 : 1)	P
One-Sucker $\times$ Burley 21	15	12	27	0.332	.95~.50
Golden Cure $\times$ Burley 21	14	16	30	0.132	.95~.50
Bright Yellow 4 $\times$ Burley 21	14	12	26	0.153	.95~.50
Hicks 2 $\times$ Burley 21	9	7	16	0.250	.95~.50
NFT 706 $\times$ Burley 21	11	12	23	0.043	.95~.50

Table 2. Segregation for resistance to wildfire disease in haploid tobacco plants emerged from anthers of the F_1 hybrids

Cross combination	Resistant	Susceptible	No. of plants	χ^2 (1 : 1)	P
One-Sucker $\times$ Burley 21	15	11	26	0.614	.50~.20
Golden Cure $\times$ Burley 21	16	14	30	0.132	.95~.50
Bright Yellow 4 $\times$ Burley 21	15	11	26	0.614	.50~.20
Hicks 2 $\times$ Burley 21	10	6	16	1.0	.50~.20
NFT 706 $\times$ Burley 21	9	14	23	1.086	.50~.20

Since the resistance to wildfire disease is controlled by a single dominant gene, a 1 resistant : 1 susceptible ratio would be expected in haploid plants as in TMV-resistance. Segregation for resistance to wildfire disease in haploid plants was also in agreement with the expected a 1 : 1 ratio (Table 2).

Segregation for leaf color (white) character in haploid tobacco plants

The white character could be observed in some of haploid plants after they had flowered. In general, it was difficult to distinguish the white character of plants from normal green ones under greenhouse conditions. However, with the approach of maturity, the stalk of haploid plants carrying the genes for the white character lost much of green. These changes were more apparent in haploid plants from the F_1 hybrid NFT 706 $\times$ Burley 21 than in those from the other F_1 hybrids.

Two double recessive genes are responsible for the white character. It is then expected that a ratio of green : white would be a 3 : 1. As is evident in Table 3, the observed ratio of green to white in those from the five F_1 hybrids agreed with the expected 3 : 1 ratio. A deficiency in numbers of the white character of plants was not observed.

Table 3. Segregation for leaf color (white) character in haploid tobacco plants emerged from anthers of the F_1 hybrids

Cross combination	Green	White	No. of plants	χ^2 (3 : 1)	P
One-Sucker × Burley 21	26	4	30	2.177	.20~.10
Golden Cure × Burley 21	25	5	30	1.111	.50~.20
Bright Yellow 4 × Burley 21	20	6	26	0.052	.95~.50
Hicks 2 × Burley 21	14	3	17	0.49	.50~.20
NFT 706 × Burley 21	21	4	25	1.08	.50~.20

Segregation for leafbase shape character in haploid tobacco plants

Leaf shape character is much complicated, but two distinctive types of leafbase shapes could be observed in haploid plants: petiolate and sessile leaves. In general, the leafbase shape can either be petiolate or sessile with varying development of wings. Its differences became apparent when the largest leaf reached 15 to 25cm in length.

Taking the results of VAN DER VEEN(1957) into consideration, it could be easily expected that haploid plants from the F_1 hybrid NFT 706 (ptptdpdbrbr) × Burley 21 (ptptPdPdBrBr) segregate for the sessile and petiolate leaves in a ratio of 1 : 1 as stated in discussion. However, those from the F_1 hybrids Bright Yellow 4 or Hicks 2 (PtPt dpdBrBr) × Burley 21 (ptptPdPdBrBr) segregated for the sessile and petiolate leaves despite

of the fact that the F_2 plants in the same cross had all the sessile leaves. In supporting of this observation, it was reported by KADOTANI and AYABE(1971) that PtPdBr had tendency to produce the petiolate leaves in haploid plants from the F_1 hybrid Hicks 2 × Cocker 139.

Fig. 1. Leafbase shape of comparable age from haploid tobacco plants emerged from anthers of the F_1 hybrid bright yellow 4 × Burleys 21.

A petiolate (left) and a sessile leaf (right).

Fig. 2. Leafbase shape of comparable age from haploid tobacco plants emerged from anthers of the F_1 hybrid NFT 706 × Burley 21.

A petiolate leaf with short petiole (left) and with long petiole (middle). A sessile leaf (right).

Therefore, it seems reasonable to postulate that haploid plants having the petiolate leaves observed here is PtPdBr. Then, 3 sessile : 1 petiolate would be expected in haploid plants from the F_1 hybrids Bright Yellow 4 or Hicks 2 $\times$ Burley 21 as described in discussion. The sessile and petiolate leaves of haploid plants are shown in Figs. 1 and 2.

Table 4. Segregation for leafbase shape character in haploid tobacco plants emerged from anthers of the F_1 hybrids

Cross combination	Sessile	Petiolate	No. of plants	χ^2	P
Bright Yellow 4 $\times$ Burley 21	21	6	27	0.111 (3 : 1)	.95~.50
Hicks 2 $\times$ Burley 21	12	5	17	0.176 (3 : 1)	.95~.50
NFT 706 $\times$ Burley 21	11	14	25	0.36 (1 : 1)	.95~.50

As given in Table 4, segregation for the sessile and petiolate in haploid plants from the F_1 hybrids, Bright Yellow 4 or Hicks 2 $\times$ Burley 21 and NFT 706 $\times$ Burley 21, was obviously in good agreement with the expectation. However, those from the F_1 hybrid Golden Cure $\times$ Burley 21 had all the sessile leaves. Haploid plants from the F_1 hybrid One-Sucker $\times$ Burley 21 also segregated for the sessile and petiolate leaves in a ratio approximating 3 : 1. However, its value was not summarised in Table 4 because the genotype for One-Sucker was not designated.

Leaf shape index

The leaf shape index was calculated in relation to the leaf shape. The index values were ranged from 0.4 to 0.7 in haploid plants from the F_1 hybrid NFT 706 $\times$ Burley 21, while from 0.3 to 0.5 in those from the two F_1 hybrids, One-Sucker $\times$ Burley 21 and Golden Cure $\times$ Burley 21, and from 0.2 to 0.5 in those from the F_1 hybrid Bright Yellow 4 $\times$ Burley 21. As indicated in these results, haploid plants from the F_1 hybrid NFT 706 $\times$ Burley 21 produced broader leaves than those from the other F_1 hybrids.

The ratio of the petiole to the leaf length

The petiolate leaves were variable in the length of petiole. The ratio of the petiole/the leaf length was calculated in an attempt to account for the morphological differences in

the petiolate leaves. This value was ranged from 0.1 to 0.12 in haploid plants from the two F_1 hybrids, Bright Yellow 4 $\times$ Burley 21 and Hicks 2 $\times$ Burley 21. On the contrary, it varied between 0.05 and 0.1 in those from the F_1 hybrid NFT 706 $\times$ Burley 21.

Segregation of the F_2 generation in the cross of Hicks 2 $\times$ Burley 21 for the resistance to TMV and wildfire diseases, and the leaf color (white) character

Segregation for those characters was in agreement with the expectations as summarised in Table 5. There were no remarkable differences between normal diploid and pollen-derived haploid plants in reaction to both TMV and wildfire diseases and in appearance of the white character. Regarding the shapes of the local lesion induced by TMV infection or the halo caused by *P. tabaci*, no differences could be observed between diploid and haploid plants even under variable conditions.

Table 5. Segregation for resistance to TMV and wildfire diseases, and leaf color (white) character of the F_2 plants in the cross of Hicks 2 $\times$ Burley 21

		Total No. of plants	χ^2	P
TMV	Resistant	50	2.221 (3 : 1)	.20~.10
	Susceptible	10		
Wildfire	Resistant	33	2.16 (3 : 1)	.20~.10
	Susceptible	17		
Leaf color	Green	56	0.01 (15 : 1)	.99~.95
	White	4		

Verification of haploid in tobacco plants growing in soil

Of thirty-one plants, growing in soil, from the F_1 hybrid Golden Cure $\times$ Burley 21, only one was diploid. However, all of the plants from the other four F_1 hybrids, One-Sucker $\times$ Burley 21, Bright Yellow 4 $\times$ Burley 21, Hicks 2 $\times$ Burley 21 and NFT 706 $\times$ Burley 21, were verified to be haploid.

Discussion

In previous work (NAKATA 1969, 1971), segregation for albino and green in haploid plants emerged from anthers of the F_1 hybrid *N. tabacum* cv Hicks 2 $\times$ *N. tabacum* bearing *Ws* (pbg) gene from *N. plumbaginifolia*, was ob-

served. Also, BURK (1970) reported the segregation of green and yellow haploid seedlings from sulphur tobacco mutants. However, these results were obtained with plantlets growing on medium. From the practical standpoint of view, therefore, it should be made clear whether the heritable characters may segregate with the expected ratios in haploid plants growing in soil.

As shown in Tables 1 and 2, it could be demonstrated that segregation for resistance to TMV and wildfire diseases was in satisfactory agreement with the expected ratio. The inheritance of resistance to these diseases is monogenic dominant. However, it is generally accepted that wildfire resistant tobacco plants show wildfire symptoms if inoculum higher than 10^7 /mL is applied to the leaves. Therefore, inoculation was always carried out using inoculum diluted at the concentration of 10^7 /mL.

For character which is governed by two double recessive genes, the white character was chosen. As given in Table 3, haploid plants from the five F_1 hybrids segregated for green and white in agreement with the expected ratio. In addition, it should be noted that no differences could be observed between diploid and haploid plants developed from pollen grains of the F_1 hybrid in reaction to infection with either TMV or *P. tabaci* and in appearance of the white character (Tables 1, 2, 3 and 5).

Many of the economic characters, being polygenic, are complex in inheritance. Therefore, more complex heritable character such as the leafbase shape was chosen for study. As stated previously, CLAUSEN and CAMERON (1944) reported the effects of three allelic pairs: Pt, Pd, Br on the leaf shape in tobacco. It was, further, shown by VAN DER VEEN (1957) and HUMPHREY *et al.* (1964) that Pt has twice the effect of Pd. In addition, these genes produced multiple effects upon the leaf (VAN DER VEEN and BINK 1961). However, all of these studies were done with diploid tobacco plants. Therefore, the studies of segregation for the leafbase shape in haploid plants were conducted in comparison with those in diploid tobacco plants.

The following genotypes, PtPd Ptpd ptPd

ptpd, but Br in common, would be expected to occur with a 1:1:1:1 ratio, on the basis of equal probabilities for possible combinations, when haploid plants were obtained from the F_1 hybrid Bright Yellow 4 or Hicks 2 (PtPt-pdpdBrBr) × Burley 21 (ptptPdPdBrBr). Similarly, the genotypes, PdBr Pdbr pdBr pdbr, but pt in common, would be expected in those from the F_1 hybrid NFT 706 (ptptpdpdbrbr) × Burley 21 (ptptPdPdBrBr).

The question would be arisen as to why PtPdBr produced the petiolate leaves in haploid plants despite of the fact that PtPtPdPdBrBr had the sessile leaves in diploid plants. A possible explanation for this is as follows. It should be noted that, in addition to the fact that haploid plants have narrow leaves as compared with those of diploid plants, Pt and Pd produce narrow wings. Thus, it would be reasonable to suppose that PtPdBr possesses the petiolate leaves resulting from very narrow wings or lack of wings.

On the other hand, VAN DER VEEN (1957) classified the F_2 plants in the cross HG × KE, similar to NFT 706 × Burley 21 with respect to the leaf shape character, into the nine phenotypes corresponding to the nine genotypes of a two-factor segregation. In comparing the leafbase shapes of haploid plants with those of diploid plants, an analogy was observed. It could be thus inferred from phenotypes that, of the two petiolate haploid plants, one with a short petiole (left in Fig. 2) was pdbr and the other with a long petiole (right in Fig. 2) was Pdbr, but pt in common.

Regarding the ratio of the petiole to the leaf length, the petiolate haploid plants from the F_1 hybrid NFT 706 × Burley 21 had values of 0.05 to 0.1, while those from the F_1 hybrid Bright Yellow 4 × Burley 21 exhibited from 0.1 to 0.12. These results were in close agreement with those of VAN DER VEEN (1957) reported that in going from pdpd to PdPd or from ptpt to PtPt the petioles become longer.

In application of this technique to gene analysis, difficulty would be encountered when any competition exists among pollen grains for formation of haploid plants. However, technical advances would overcome these problems. In general, the whitish plantlets are likely to be dedifferentiated into callus on

medium. Therefore, the concentration of constituents of medium in this study was lowered to an extent that does not cause dedifferentiation of plantlets into callus. With this medium, haploid plantlets could be easily developed from pollen grains of the F_1 hybrids, but only a few plantlets were formed from commercial varieties which were regarded as homozygous. It seems that the former vigorous production of plantlets may be due to heterosis.

The whitish plantlets rarely emerged from anthers. However, a ratio of the whitish to the green plantlets on medium was not constant. As already mentioned, identification of the white character becomes possible after plants flowered except for those from the F_1 hybrid NFT 706×Burley 21. Therefore, it is quite difficult to discern the whitish plantlets of the genotype responsible for the white character on medium.

It is of particular interest to know how the whitish plantlets emerged from anthers in culture. More recently, SUNDERLAND and WICKS (1969, 1971) found that only vegetative nucleus of pollen grain divided in many instances of culture. Furthermore, DEVREUX (1970) suggested that the whitish plantlets might be originated from the generative nucleus. However, the whitish plantlets formation is a rare events. This indicates that the occurrence of the whitish plantlets has no effects on the segregating ratios.

The use of this technique as a means for rapid development of disease resistant varieties is being examined by OKA and NAKAMURA (personal communication). Also, haploid tobacco plants of varying alkaloid levels were found by BURK (personal communication). The present report gave additional evidences for the absence of competition among pollen grains and thus proved that this technique would be contributed to gene analysis as well as plant breeding.

Summary

In order to test whether any competition exists among pollen grains for haploid plant formation, the segregating ratios for resistant and susceptible to TMV and wildfire diseases, for green and white leaf colors, and for sessile

and petiolate leaf shapes in haploid tobacco plants developed from pollen grains of the five F_1 hybrids, were compared with the expected ratios.

Haploid plants were obtained from the five F_1 hybrids among cultivated varieties of *Nicotiana tabacum* L.; One-Sucker×Burley 21, Golden Cure×Burley 21, Bright Yellow 4×Burley 21, Hicks 2×Burley 21, and NFT 706×Burley 21.

Segregation for resistance to TMV and wildfire diseases in haploid plants was obviously in satisfactory agreement with the expected 1:1 ratio. Also, haploid plants segregated for green and white in the 3:1 expectation.

Regarding the leafbase shape character, those from the two F_1 hybrids, Bright Yellow 4×Burley 21 and Hicks 2×Burley 21 segregated into the expected 3:1 ratio for sessile and petiolate. On the other hand, those from the F_1 hybrid NFT 706×Burley 21 segregated in the expected 1:1 ratio.

Plantlet formation was equally achieved in all of the five F_1 hybrids. The occurrence of the whitish plantlets was rarely found on medium, but had no effects on the segregating ratios.

These results conclusively demonstrated, on the basis of the absence of competition among pollen grains, that this technique is applicable to gene analysis.

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Literature Cited

- BURK, L. G. 1970. Green and light-yellow haploid seedlings from anthers of sulfur tobacco. *Jour. Hered.* 61: 279.
 CLAUSEN, R.E. and D.R. CAMERON 1944. Inheritance in *Nicotiana tabacum*. XVIII. Monosomic analysis.

- Genetics 29 : 447~477.
- CLAYTON, E. E. 1947. A wildfire resistant tobacco. Jour. Hered. 38 : 35~40.
- DEVREUX, M. 1970. New possibilities for the in vitro cultivation of plant cells. Scientific and Technical Review of the European Communities IX : 105~110.
- EGUCHI, K. 1971. On major genes controlling leaf shapes in flue-cured tobacco. Bull. Iwata Tobacco Exp. Sta. 3 : 35~44.
- HEGSTAD, H. E., E. E. CLAYTON, M. O. NEAS and H. A. SKOOG 1960. Development of Burley 21 the first wildfire-resistant tobacco variety, including results of variety trials. Tenn. Univ. Agric. Expt. Sta., Bull. 321 : 1~51.
- HENIKA, F. A. 1932. The inheritance of the white burley character in tobacco. J. Agric. Res. 44 : 477~493.
- HOLMES, F. O. 1938. Inheritance of resistance to tobacco mosaic disease in tobacco. Phytopath. 28 : 553~561.
- HUMPHREY, A. B., D. F. MATZINGER and T. J. MANN 1964. Inheritance of leaf shape in flue-cured tobacco (*Nicotiana tabacum* L.). Heredity 19 : 615~628.
- KADOTANI, K. and T. AYABE 1971. The selection of the main characters in haploid generation tobacco. Ann. Repr. Iwata Tobacco Exp. Sta. pp. 21~24.
- NAKATA, K. 1969. The effects of the inter-pollen-grain competition on the differentiation of haploid by the anther culture of tobacco. Japan. J. Breed. 19. Suppl. 2 : 123~124.
- 1971. Competition among pollen grains for haploid tobacco plant formation by anther culture. 1. Analysis with leaf color character. Japan. J. Breed. 21 : 29~34.
- and T. KURIHARA 1971. Competition among pollen grains for haploid tobacco plant formation by anther culture. II. Analysis with resistance to TMV and wildfire diseases, and leaf color characters etc. Japan. J. Breed. 21. Suppl. 1 : 124~125.
- SUNDERLAND, N. and F. M. WICKS 1969. Cultivation of haploid plants from tobacco pollen. Nature (London) 224 : 1227~1229.
- and ——— 1971. Embryoid formation in pollen grains of *Nicotiana tabacum*. J. Experimental Botany 22 : 213~226.
- VAN DER VEEN, J. H. 1957. Studies on the inheritance of leaf shape in *Nicotiana tabacum* L. Thesis, Agric. Univ. Wageningen, The Netherlands.
- and J. P. M. BINK 1961. Multiple effects of the leaf shape allele Pt in *Nicotiana tabacum* L. Genetica 32 : 35~50.
- VALLEAU, W. D. 1949. Breeding tobacco for disease resistance. Economic Botany 6 : 69~102.

薬培養によるタバコ半数体形成における花粉間競合

II. タバコモザイクウイルス (TMV) および野火病抵抗性、葉色形質ならびに葉柄の有無による解析

中 田 和 男・栗 原 恒

(秦野たばこ試験場)

薬培養による半数体形成において、本技術が遺伝子分析に有要な手段となり得るかを検討するため、本実験を行なった。

Nicotiana tabacum L. の栽培品種間の五つの雑種 F_1 すなわち One-Sucker×Burley 21, Golden Cure×Burley 21, Bright Yellow 4×Burley 21, Hicks 2×Burley 21 および NFT 706×Burley 21 から育成した半数体における TMV および野火病抵抗性、葉色形質ならびに葉柄の有無の分離比を期待値と比較した。

TMV および野火病抵抗性のいずれも期待値 (1 : 1) に対して、高い適中率を示した。またこれらの半数体は、

緑色と白色形質を 3 : 1 の期待値で分離した。

葉柄の有無に関しては、Bright Yellow 4×Burley 21 および Hicks 2×Burley 21 からでは、無柄 : 有柄を期待値の 3 : 1 で分離したが、NFT 706×Burley 21 では、1 : 1 の期待値で分離した。

薬培養による幼植物の形成は、五つの F_1 の雑種において、同じように行なわれた。白色性の幼植物の形成が見られたが、分離比に影響を及ぼさなかった。

これらの結果から花粉間に競合が存在しないことが明らかにされ、本技術が遺伝子分析に用いられ得ることが判明した。