PERFORMANCE OF ANTHER-DERIVED DIHAPLOIDS IN MARYLAND TOBACCO[1]

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TOBACCO SCIENCE

The objectives of this study were to develop anther-derived dihaploid lines from two Maryland tobacco (*Nicotiana tabacum* L.) cultivars, 'MD 609' and 'MD 341', and to compare the performance of the dihaploids with their parental sources. Haploid production per anther was 2.8 and 1.0 for MD 609 and MD 341, respectively. The average dihaploid production per haploid was approximately 4.0 for both populations. Seventeen and 23 dihaploid lines from MD 609 and MD 341, respectively, were grown in the field and then evaluated with their parental cultivars for 11 agronomic and chemical traits in a 2-year study (1986 and 1987). Six weekly growth increments were measured for the MD 341 dihaploid population in 1986. Parental genotypes appeared to have a significant effect on dihaploid performance. MD 609 dihaploids demonstrated no significant yield reductions, but they were lower in price and quality index, had fewer leaves, and flowered later than the MD 609 cultivar. MD 341 dihaploids were 5.6% lower yielding, had longer internodes, and flowered later than the MD 341 cultivar. Average weekly growth rates for MD 341 and its dihaploids were similar throughout the growing season. Significant variation for several traits was observed among the dihaploids in both populations. Therefore, the small reduction in performance of anther-derived dihaploids in the two Maryland tobacco populations suggests that anther culture is a viable technique for developing homozygous germplasm lines and for genetic studies in Maryland tobacco.

Additional key words: *Nicotiana tabacum* L., tissue culture, haploids.

INTRODUCTION

Since Guha & Maheshwari (15) reported on the production of haploid plantlets by aseptic culture of anthers from *Datura*, a number of studies have been conducted with tobacco (*Nicotiana tabacum* L.) to determine the value of this technique for basic biological investigations and breeding for improved germplasm. Anther-derived dihaploids from burley tobacco have lower total alkaloids, shorter leaves, reduced yields, and later flowering times than inbred or conventionally derived pure lines (8,9,10,18). Similar investigations with flue-cured tobacco suggest that the performance of anther-derived dihaploids is inferior to parental lines, and that the reduction in performance is greater than what is observed for burley tobacco (1,7). Significant variation has been observed among dihaploid lines derived from a flue-cured hybrid (5) and among dihaploid lines derived from pure-line cultivars of burley (8,10) and flue-cured (1,7) tobaccos.

Burk et al. (6) suggested an alternative to anther culture in which maternal haploids are produced from crosses involving *N. africana* Merxmüller as the pollen parent. For burley tobacco, Nielsen & Collins (22) found that the levels of performance of androgenetic doubled haploids (ADH) and gynogenetic doubled haploids (GDH) are influenced by the source plant of the parental cultivar. Wernsman et al. (26) reported that maternally-derived doubled haploid lines are agronomically superior to ADH lines and they more closely resemble parental cultivars.

To date, dihaploid lines of Maryland tobacco derived from either ADH or GDH have not been evaluated. Therefore, the objectives of this study were to develop anther-derived dihaploid lines from two pure-line cultivars, and to compare the performance of the dihaploids with their parental cultivars.

MATERIALS AND METHODS

Dihaploid Production

Maryland tobacco cultivars, 'MD 609' and 'MD 341', were used for the production of two populations of anther-derived dihaploids. Young flower buds from four greenhouse-grown plants of each cultivar (P1, P2, P3, & P4 from MD 341; and P2, P3, P4, & P5 from MD 609) were collected at Stage 2 (23). The anthers were cultured on Nitsch medium (23), and haploid plantlets were rooted on medium described by Kasperbauer & Wilson (19). Plants that produced compact flower heads with sterile flowers were assumed to be haploid. Dihaploids were produced from the culture of haploid midveins (16), and they were grown to maturity for seed production.

Field Studies

The dihaploids were evaluated under field conditions for two years (1986 and 1987) at the Upper Marlboro Facility, Central Maryland Research and Education Center. The soil type was a Monmouth fine sandy loam which is a deep, well-drained soil (Typic Hapludult).

The MD 341 population consisted of 25 entries. They were two entries of MD 341 (selfed seed from two parental plants, P1 and P2), 15 dihaploid lines derived from four haploids from P1, and eight dihaploid lines derived from two haploids from P2. The MD 609 population consisted of 19 entries. They were two entries of MD 609 (selfed seed from two parental plants, P4 and P5), six dihaploid lines derived from two haploids from P4, and 11 dihaploid lines derived from five haploids from P5. For both populations, the 13 haploids produced a total of 40 dihaploids. For each year, the entries from each population were planted separately in

Table 1. Haploid and dihaploid production from two cultivars of Maryland tobacco.

Plant number	Anthers cultured	Haploids produced	Haploids per anther	Surviving haploids	Haploids producing dihaploids	Dihaploids produced	Dihaploids per haploid
	number						
MD 341 Cultivar							
P1[a]	140	58	0.4	28	10	51	5.1
P2[a]	110	97	0.9	52	11	71	6.5
P3	145	51	2.1	27	6	13	2.2
P4	55	32	0.6	19	10	33	3.3
Total/mean	450	238	1.0	126	37	168	4.3
MD 609 Cultivar							
P2	15	73	4.9	34	7	11	1.6
P3	40	69	1.7	40	7	29	4.1
P4[a]	50	100	2.0	54	9	43	4.8
P5[a]	40	102	2.6	51	12	67	5.6
Total/mean	145	344	2.8	179	35	150	4.0

[a]Dihaploids from plants 1 and 2 of the MD 341 cultivar and from plants 4 and 5 of the MD 609 cultivar were used in the field evaluations.

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randomized complete block (RCB) designs with three replications. Each plot consisted of a single row of 20 plants. Rows were 102 cm apart and plants were spaced 61 cm within a row.

Dihaploid lines were compared with their parental cultivars for 11 chemical and agronomic traits. A detailed description of data collection was given by McConnell (20). At approximately three-day intervals during the flowering period, inflorescences with at least one open flower were counted. Determinations were made of the average number of days after transplanting that was required for 50% of the plants in each plot to flower (time to flower). Both populations were topped at developmental Stage 7, defined as mid-flower (4).

After removing the end or border plants from each plot, fifteen competitive plants in 1986 and 10 competitive plants in 1987 were harvested by cutting the entire stalk. After curing, the leaves were removed from the stalks and sorted into grades according to stalk position, quality, and color. Yield and average price/kg of the cured leaves were calculated from the plot weight and estimated price of the grades. Quality index was calculated for each plot as described by Mulchi (21). Plant height at maturity and the number of leaves per plant were recorded for 10 stalks after the stripping and grading operations. The average internode length for each plant was obtained by dividing the height of the plant by its number of leaves.

A weighted composite sample was taken of cured leaves from the various grades from each plot. This sample was used for chemical determinations. Total alkaloids were determined by steam distillation (14), and total nitrogen was determined by the Ranker Semi-Micro Kjeldahl method (13).

Data on burning characteristics (burn time) were collected on a 15-leaf and 10-leaf sample from each plot in 1986 and 1987, respectively. One leaf was obtained from each harvested plant at the midstalk position. The samples were preconditioned at 20°C and 75% relative humidity for 24 hours before burning. Burn time was determined after igniting a section of the mid-lamina of the leaf with a small electric coil. Average burn time per leaf was calculated for each plot. Data on filling capacity were obtained from another section of the same leaf that was used to determine burn time. The procedure outlined by Artho et al. (2) was followed.

For the MD 341 dihaploid population in 1986, six growth increments were calculated from weekly plant height measurements beginning 28 days after transplanting. The same 10 competitive plants in each plot were measured each week.

For each trait measured, a two-year combined analysis of variance appropriate for an RCB was conducted. Single degree of freedom comparisons were calculated for parent vs. dihaploids, and dihaploids from one parent plant vs. dihaploids from the other parent plant (P1 DH vs. P2 DH for MD 341 population and P4 DH vs. P5 DH for MD 609 population). Also, the significance of the variation among the dihaploids from a specific parent cultivar was calculated.

RESULTS AND DISCUSSION

Dihaploid Production

MD 609 and MD 341 cultivars produced 179 and 126 surviving haploids, respectively (**Table 1**). This resulted in a survival rate of approximately 52% for all haploids cultured. The number of dihaploids produced per haploid was similar for each cultivar; however, variation for haploid and dihaploid production among the source plants in each cultivar was observed. The number of dihaploids produced per haploid ranged from 1.6 to 5.6 for MD 609 and 2.2 to 6.5 for MD 341 (**Table 1**). Some plantlets died during each step of the haploid and dihaploid production process. Death was due to a number of reasons, but the major factors were a failure of the plantlets to develop a root system and a low survival rate after plantlets were transplanted to peat pellets or plastic pots. A high proportion of the plantlets demonstrated a condition known as vitrification, which is characterized by a water-soaked appearance of the tissue, abnormal development of the stomates, and low epicuticular wax formation. This condition frequently resulted in plants that did not survive transplanting (12).

Previous reports of plantlet (haploid) production per anther in tobacco have ranged from 0 to 50+ (16), 1 to 100 (22), and 0 to 139 (17). Kasperbauer & Collins (17) reported that plantlet production per anther ranged from an average of 2.2 for flue-cured types to 8.9 for dark-fired types of tobacco. The haploid production level in our study was similar to their flue-cured data with MD 609 and MD 341 averaging 2.8 and 1.0 haploids per anther, respectively (**Table 1**).

Table 2. Means and specific contrasts for agronomic and quality traits of 17 dihaploids and the MD 609 parent combined over two years, 1986-87.

Treatment	Yield	Price	Quality index	Plant height	Leaves	Internode length	Time to Flower	Total alkaloids	Total nitrogen	Burn Time	Filling Capacity
	kg ha^{-1}	$ kg^{-1}	5-100	cm	no.	cm	days	%	%	sec.	cm^3 g^{-1}
Means											
All entries	2,516	2.90	49.8	91.9	22.5	4.10	75.0	3.02	3.81	10.7	8.1
MD 609 parent[a]	2,526	3.00	58.0	92.8	23.2	4.01	71.8	2.94	3.71	11.6	8.5
All dihaploids (DH)	2,515	2.89	48.9	91.9	22.4	4.11	75.4	3.04	3.82	10.6	8.0
Range: High	2,743	2.96	53.8	97.7	23.3	4.40	81.3	3.23	4.05	12.6	9.1
Low	2,381	2.80	41.8	87.2	21.6	3.89	71.0	2.76	3.54	8.1	7.0
P4 dihaploids[b]	2,545	2.88	49.4	91.8	22.5	4.10	77.5	3.10	3.78	10.5	8.3
P5 dihaploids[b]	2,499	2.89	48.6	91.9	22.4	4.12	74.3	3.00	3.84	10.7	7.9
Contrasts											
Among all entries	*		*	*		**	**				
Parent vs. DH		*	**		*		**				
P4 DH vs. P5 DH							**				*
Among P4 DH	**						**				
Among P5 DH				**		**	**				
Yearly Means											
1986	2,559	3.17	62.8	94.4	21.7	4.36	70.2	2.24	3.41	16.7	7.2
1987	2,474	2.63	36.8	89.5	23.3	3.84	76.2	3.81	4.21	4.8	9.0
1986 vs. 1987		**	*		*		*	**	**	**	*
E x Y interaction	*			*	**		**				*

*, ** Significant at P = 0.05 and 0.01, respectively.

[a]Average of the two MD 609 entries.

[b]Six dihaploids from plant 4 (P4) and 11 dihaploids from plant 5 (P5) were evaluated.

Table 3. Means and specific contrasts for agronomic and quality traits of 23 dihaploids and the MD 341 parent combined over two years, 1986-87.

Treatment	Yield	Price	Quality index	Plant height	Leaves	Internode length	Time to Flower	Total alkaloids	Total nitrogen	Burn Time	Filling Capacity
	kg ha^{-1}	\$ kg^{-1}	5-100	cm	no.	cm	days	%	%	sec.	cm^3 g^{-1}
Means											
All entries	2,636	2.90	44.7	92.1	25.6	3.60	68.6	3.09	3.24	11.1	8.7
MD 341 parent[a]	2,781	2.94	47.0	92.2	26.5	3.48	65.9	3.09	3.17	11.4	8.8
All dihaploids (DH)	2,624	2.90	44.5	92.0	25.5	3.61	68.8	3.10	3.24	11.0	8.8
Range: High	2,789	2.98	50.8	95.0	27.0	3.79	78.2	3.52	3.41	13.6	9.2
Low	2,473	2.79	36.0	89.2	24.0	3.44	64.4	2.80	3.07	8.4	8.3
P1 dihaploids[b]	2,612	2.91	45.1	92.3	25.2	3.67	67.5	3.10	3.26	10.8	8.7
P2 dihaploids[b]	2,646	2.88	43.4	91.6	26.2	3.49	71.4	3.09	3.21	11.5	8.9
Contrasts											
Among all entries	**	**	**		**	**	**	**		*	
Parent vs. DH	**					*	**				
P1 DH vs. P2 DH					**	**	**				**
Among P1 DH		*	**				*	**			
Among P2 DH	**	**	*				**	**			
Yearly Means											
1986	2,770	3.05	48.0	95.6	26.2	3.65	70.1	2.41	2.76	15.8	8.9
1987	2,502	2.75	41.4	88.6	25.0	3.51	67.1	3.78	3.72	6.4	8.6
1986 vs. 1987	**				*			**	**	*	
E x Y interaction						**	**	**			

*, ** Significant at P = 0.05 and 0.01, respectively.
[a]Average of the two MD 341 entries.
[b]Fifteen dihaploids from plant 1 (P1) and 8 dihaploids from plant 2 (P2) were evaluated.

Field Studies

The MD 609 population was higher in price and quality index, had fewer leaves, flowered earlier, produced lower levels of total alkaloids and total nitrogen, burned better, and had a lower filling capacity in 1986 than in 1987 (**Table 2**). The MD 341 population produced a higher yield and more leaves in 1986 than in 1987, and its yearly means for chemical content and burn time were similar to the MD 609 population (**Table 3**). The entry x year interaction was significant for five traits in the MD 609 population and for three traits in the MD 341 population. The significance of these interactions was due primarily to differences in the magnitude of yearly responses, except for yield in the MD 609 population, which was due to changes in the ranking of the dihaploids over the two years.

The 19 entries in the MD 609 population varied significantly for yield, quality index, plant height, internode length, and time to flowering (**Table 2**). Generally, the dihaploids were lower in price and quality index, had fewer leaves, and flowered later than the MD 609 parent. For certain traits, significant differences between the dihaploids derived from the two different MD 609 parent plants (P4 and P5) were observed. The P5 dihaploids were earlier flowering and had a lower filling capacity than the P4 dihaploids. Significant variation was observed among the P4 and P5 dihaploids for average time to flowering. In addition, the P4 dihaploids varied significantly for yield, and the P5 dihaploids varied significantly for plant height and internode length.

In general, there appeared to be greater variation in the MD 341 population than in the MD 609 population (**Table 3**). Among all entries, significant variation was found for eight agronomic and chemical traits. The 23 dihaploids were lower yielding, had longer internodes, and flowered later than the MD 341 parent. The 15 dihaploids from plant 1 of MD 341 averaged more leaves, had shorter internodes, flowered earlier, and exhibited a lower filling capacity than the 8 dihaploids from plant 2 (P1 DH vs. P2 DH contrast, **Table 3**). Variation among the dihaploids from both parent plants was significant for price, quality index, time to flowering, and total alkaloids. In addition, significant differences were observed among the P2 dihaploids for yield.

As indicated by the non-significant Parent vs. DH comparisons in **Table 4**, weekly growth rates for the MD 341 dihaploids were similar to the parent cultivar. The P1 dihaploids grew faster than the P2 dihaploids during the time period of 42 to 63 days after transplanting. Also, significant variations in growth rate were found among the P2 dihaploids for two growth periods (28-35 and 56-63 days). In contrast to this study, Arcia et al. (1) found that flue-cured dihaploids grew more slowly than the parents.

The lack of reduction in yield for the MD 609 dihaploids was similar to what was reported in one study on burley tobacco (10). The 5.6% yield reduction for the MD 341 dihaploids was similar to the 4.8% decrease that was reported in another study on burley (8), but it was considerably less than the 8 to 20% reductions reported in other studies on burley (9,22,26) and flue-cured (1) tobaccos. Also, the significant variation observed among MD 609 and MD 341 dihaploids for several agronomic traits is similar to previous reports for other types of tobacco (1,7,8).

The source genotype influences the reduction in vigor observed in the resultant dihaploids (10). Although the average yield losses of the dihaploids compared to the parental lines were small (11 and 157 kg ha^{-1} for the MD 609 and MD 341 dihaploids, respectively), these

Table 4. Weekly growth increments and specific contrasts for 23 dihaploids and the MD 341 parent, 1986.

Treatments	Days after transplanting 28-35	35-42	42-49	49-56	56-63	63-70
	cm					
Means						
All entries	16.3	15.5	22.0	27.1	43.4	26.3
MD 341 parent	16.4	14.8	22.6	26.8	46.4	24.9
All dihaploids (DH)	16.2	15.6	21.9	27.1	43.1	26.4
Range: High	18.8	17.5	25.7	37.2	50.8	33.5
Low	13.6	13.1	17.3	22.3	33.1	18.3
P1 dihaploids[a]	16.4	15.9	22.5	28.1	45.2	25.6
P2 dihaploids[a]	15.9	14.9	20.7	25.3	39.1	28.0
Contrasts						
Among all entries						
Parent vs. DH						
P1 DH vs. P2 DH			*	*	**	
Among P1 DH						
Among P2 DH	*				*	

*, ** Significant at P = 0.05 and 0.01, respectively.
[a]Fifteen dihaploids from plant 1 (P1) and 8 dihaploids from plant 2 (P2) were evaluated.

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reductions are still smaller than the 517 and 423 kg ha^{-1} losses observed for dihaploids from flue-cured cultivars Coker 139 and NC 95, respectively (25).

In recent years, researchers have suggested several theories to explain the reduced performance (primarily yield) of anther-derived dihaploids. These theories include the presence of residual heterozygosity in the original cultivars (10); genetic differentiation between dihaploids and their source cultivars due to changes in nuclear rather than cytoplasmic factors (3); the possibility that the present dihaploid production technique may be mutagenic (1); and amplification of DNA sequences during dihaploid formation (11). However, Reed & Wernsman (25) suggested that DNA amplification and yield losses are not strongly correlated. More recently, Reed et al. (24) suggested that there may be some specificity to the DNA amplification that occurs as a result of anther culture in tobacco.

The small reduction in performance of anther-derived dihaploids in two Maryland tobacco populations suggests that anther culture could be a viable technique for developing homozygous genetic material. However, Kasperbauer et al. (18) suggested that differences detected among doubled haploid lines may be due to somatic mutations in the haploid plant or genetic changes induced by in-vitro culture. Therefore, doubled haploids should be evaluated before they are used for further studies.

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LITERATURE CITED

1. Arcia, M. A., E. A. Wernsman, and L. G. Burk. Performance of anther-derived dihaploids and their conventionally inbred parents as lines, in F_1 hybrids, and in F_2 generations. **Crop Sci.** 18:413-418. 1978.

2. Artho, A. J., R. J. Monroe, and J. A. Weybrew. Physical characteristics of cured tobacco. I. Simplified procedures for measuring specific volume and fragility. **Tob. Sci.** 7:191-197. 1963.

3. Brown, J. S., and E. A. Wernsman. Nature of reduced productivity of anther-derived dihaploid lines of flue-cured tobacco. **Crop Sci.** 22:1-5. 1982.

4. Bruns, H. A., and M. K. Aycock, Jr. Developmental stages of Maryland tobacco with respect to management. Maryland Agric. Exp. Stn. Bull., ESB-4. 1988.

5. Burk, L. G., and J. F. Chaplin. Variation among anther-derived dihaploids from a multiple disease-resistant tobacco hybrid. **Crop Sci.** 20:334-338. 1980.

6. Burk, L. G., D. U. Gerstel, and E. A. Wernsman. Maternal haploids of *Nicotiana tabacum* L. from seed. **Science** 206:585. 1979.

7. Burk, L. G., and D. F. Matzinger. Variation among anther-derived doubled haploids from an inbred line of tobacco. **J. Hered.** 67:381-384. 1976.

8. Collins, G. B., P. D. Legg, and C. C. Litton. The use of anther derived haploids in *Nicotiana*. II. Comparison of doubled haploid lines with lines obtained by conventional breeding methods. **Tob. Sci.** 18:40-42. 1974.

9. Deaton, W. R., G. B. Collins, and M. T. Nielsen. Vigor and variation expressed by anther-derived doubled haploids of burley tobacco (*Nicotiana tabacum* L.). I. Comparison of sexual and doubled-haploid populations. **Euphytica** 35:33-40. 1986.

10. Deaton, W. R., P. D. Legg, and G. B. Collins. A comparison of burley tobacco doubled-haploid lines with their source inbred cultivars. **Theor. Appl. Genet.** 62:69-74. 1982.

11. Dhillon, S. S., E. A. Wernsman, and J. P. Miksche. Evaluation of nuclear DNA content and heterochromatin changes in anther-derived dihaploids of tobacco (*Nicotiana tabacum*) cv. Coker 139. **Can. J. Genet. Cytol.** 35:169-173. 1983.

12. Evans, D. A., I. Y. E. Chu, R. D. Hartman, and H. J. Swartz. Summary of panel discussion on phenotypic and genotypic stability of tissue cultured plants. p. 95-96. In R. H. Zimmerman, R. J. Griesbach, F. A. Hammerschlag, and R. H. Lawson (ed.). *Tissue Culture as a Plant Production System for Horticultural Crops*. Martinus Nijhoff Publ. Dordrecht, The Netherlands. 1986.

13. Gaines, T. P. Chemical methods of tobacco plant analysis. Georgia Agric. Exp. Stn. Res. Rep. No. 97. 1971.

14. Griffith, R. B. The rapid determination of total alkaloids by steam distillation. **Tob. Sci.** 1:130-137. 1957.

15. Guha, S., and S. G. Maheshwari. In vitro production of embryos from anthers of *Datura*. **Nature** 204:497. 1964.

16. Kasperbauer, M. J., and G. B. Collins. Reconstitution of diploids from leaf tissue of anther-derived haploids in tobacco. **Crop Sci.** 12:98-101. 1972.

17. Kasperbauer, M. J., and G. B. Collins. Anther-derived haploids in tobacco: Evaluation of procedures. **Crop Sci.** 14:305-307. 1974.

18. Kasperbauer, M. J., P. D. Legg, and T. G. Sutton. Growth, development, and alkaloid content of doubled haploids vs. inbreds of burley tobacco. **Crop Sci.** 23:965-969. 1983.

19. Kasperbauer, M. J., and H. M. Wilson. Haploid plant production and use. p. 33-39. In R. D. Durbin (ed.). *Nicotiana: Procedures for Experimental Use*. USDA Tech. Bull. No. 1586. 1979.

20. McConnell, M. E. Performance of anther-derived dihaploids in Maryland tobacco. M.S. Thesis. Univ. of Maryland, College Park. 1988.

21. Mulchi, C. L. Development of an index of quality for Maryland tobacco. Maryland Agric. Exp. Stn. Bull. MP 909. 1977.

22. Nielson, M. T., and G. B. Collins. Variation among androgenic and gynogenic doubled haploids of tobacco (*Nicotiana tabacum* L.). **Euphytica** 43:263-267. 1989.

23. Nitsch, J. P. Haploid plants from pollen. **Z. Pflanzenzücht**. 67:3-18. 1972.

24. Reed, S. M., J. A. Burns, and E. A. Wernsman. Cytological comparison of amplified chromosome segments in four tobacco doubled haploids. **Crop Sci.** 32:362-366. 1992.

25. Reed, S. M., and E. A. Wernsman. DNA amplification among anther-derived doubled haploid lines of tobacco and its relationship to agronomic performance. **Crop Sci.** 29:1072-1076. 1989.

26. Wernsman, E. A., D. F. Matzinger, and R. C. Rufty. Androgenetic vs. gynogenetic doubled haploids of tobacco. **Crop Sci.** 29:1151-1155. 1989.