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A Comparison of Burley Tobacco Doubled-haploid Lines with Their Source Inbred Cultivars*

W. R. Deaton, P. D. Legg and G. B. Collins
Department of Agronomy, University of Kentucky, Lexington, Kentucky (USA)

Summary. A doubled-haploid line was randomly derived from each of seven burley tobacco, *Nicotiana tabacum* L., cultivars using anther culture and leaf-midvein chromosomal doubling. The doubled-haploid lines were compared to their source inbred cultivars in two experiments for several agronomic and chemical characters. A consistent relationship between anther-derived materials and reduced vigor was not observed in these doubled-haploid lines. Significant differences between the doubled-haploid mean and their source cultivar mean were observed only for days to flower and total alkaloids. The doubled-haploid means were not significantly different from their source cultivar means for yield, plant height, and leaf number. In total, 26 of the 35 individual comparisons between a doubled-haploid line and its source cultivar were nonsignificant. Reciprocal crosses between the doubled-haploid lines and their source inbred cultivars also demonstrated no significant differences. The diallel progeny of the seven doubled-haploid lines showed a similar genetic performance to that of diallel progeny from conventional materials in previous studies. The differences observed between the doubled-haploid lines and their source inbred cultivars could be explained by residual heterozygosity. The performances of the doubled-haploid progeny could also be attributed to the presence of residual heterozygosity in the original cultivars. As tested by reciprocal crosses, cytoplasmic effects were not significantly involved in the performance of the doubled-haploid lines.

Key words: *Nicotiana tabacum* L. – Anther culture – Haploids – Diallel – Reciprocal cross

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Introduction

Anther and pollen culture techniques have made haploid production efficient, especially in Solanaceous genera such as *Nicotiana* (Collins 1977). Using one of the available methods for doubling the chromosome number of haploids, homozygous diploid lines can be rapidly produced from haploids (Jensen 1974). Doubled haploids produced by these techniques have been shown to be meiotically stable (Collins and Sadasivaiah 1972) and free of observable chromosomal aberrations (Gerstel et al. 1974).

Doubled-haploid lines have been derived from inbred tobacco (*Nicotiana tabacum* L.) sources by several investigators to determine the vigor and variation associated with haploid derivatives (Arcia et al. 1978; Burk and Matzinger 1976; Collins et al. 1973; De Paepe et al. 1977). Reduced vigor has been observed in some doubled haploids when compared to their inbred source materials. There is also evidence of variability among lines derived from the same source. Doubled haploids derived from hybrid tobacco sources have been reported to demonstrate both improved performances over midparent (Burk and Chaplin 1980) and reduced vigor relative to a similar population derived by single-seed-descent breeding (Schnell et al. 1980). Vigor reduction and variation found in doubled-haploid materials have been attributed to residual heterozygosity in inbred cultivars, cytoplasmic effects due to culturing microspores, effects from colchicine treatment, and an intrinsic mutagenic nature in the anther-culture process.

This study was conducted to determine if doubled haploids from burley tobacco demonstrate reduced vigor and to obtain information on the cause(s) of differences between doubled haploids and their source inbred cultivars.

Materials and Methods

Source Materials

Seven burley tobacco cultivars – 'Burley 1', 'Burley 21', 'Burley 49', 'Ky 16', 'Ky 41A', 'Ky 61', and 'Va 509' – were used as source materials. These cultivars were chosen to provide a rep-

resentative sample of genetic differences in burley tobacco. All had been maintained by single-plant self-pollination since their release as inbred lines. A series of doubled haploids was derived from each cultivar using anther and leaf-midvein culture techniques (Kasperbauer and Collins 1972). One randomly-selected doubled haploid from each cultivar was grown to maturity and self-pollinated to produce a total of seven lines. Five plants from each of these lines and their seven parental cultivars were planted in nursery plots. Crosses for two experiments were made with a single, randomly-selected plant from each nursery plot.

Experimental Materials and Design

Experiment 1: Reciprocal crosses were made between each doubled-haploid line and its source cultivar. Plants used to make the crosses were self-pollinated. The materials for Experiment 1 consisted of 28 entries: 14 reciprocal crosses, seven doubled-haploid lines, and seven cultivar selfed lines. The materials were evaluated in a split-plot design with three replications. The main plots were the cultivar families; and the subplots were the cultivar, the doubled-haploid line, and the reciprocal crosses.
Experiment 2: All possible crosses, excluding reciprocals, were made among the seven doubled-haploid lines. The materials for Experiment 2 consisted of 28 entries: seven parental selfed lines and 21 F_1 crosses. The materials were evaluated in a randomized complete block design with three replications.

Cultural Practices

The experiments were conducted north of Lexington, Kentucky in 1978. Experimental materials were evaluated in a single environment. Since gentoype × environment interactions are small in burly tobacco (Gupton et al. 1974), it was determined that the additional information obtained from including multiple environments would not be adequately balanced by the additional time and cost. The experimental area had a soil type of Maury silt loam and had previously been in bluegrass sod for two years. Sufficient fertilizer was applied prior to transplanting to bring the available N level to 112 kg/ha and the P and K levels to 336 kg/ha. All plots were single rows consisting of 25 plants spaced 46 cm apart with a spacing between rows of 107 cm. Other cultural practices were the same as those normally followed in the production of burley tobacco.

Data Collection

Data were obtained for the following characters:

i) Days to flower – When the first plant within a plot began to flower, a cumulative count of the plants in flower was taken every two or three days. The number of days from transplanting to the date when 50% of the plants flowered was determined and used for analysis.

ii) Plant height – After 50% of the plants within a plot had flowered, the inflorescences were removed at a point two leaves below the lowest floral branch. The height in centimeters from ground level to the point of topping was measured on ten competitive plants. Plot means were used for analysis.

iii) Leaf length – The length in centimeters of the eighth leaf from the bottom on each of ten plants was measured. Plot means were calculated for analysis (Experiment 2 only).

iv) Leaf width – The width in centimeters was measured for the same leaves used to obtain leaf length (Experiment 2 only).

v) Yield – Twenty plants per plot were stalk harvested and air cured. After the leaves were removed from the stalks, the weight of cured leaves per plot was obtained. Whole plot weights were converted to grams of cured leaves per plant for analysis.

vi) Leaf number – The number of leaf nodes on ten of the harvested and stripped stalks was counted. Plot means were used for analysis.

vii) Total alkaloids – Leaf samples from each plot were used for determination of the percent total alkaloids on a dry weight basis by the method of Harvey et al. (1969) using an autoanalyzer.

Statistical Analysis

The data from the two experiments were statistically analyzed by analyses of variance.
Experiment 1: Differences among the seven cultivar family main plots were tested by error a (replications × cultivar families). The sums of squares associated with the differences among sub-plot lines were partitioned into three orthogonal contrast: cultivars vs. doubled haploids (C vs. DH), parents vs. crosses (P vs. F_1), and reciprocal effects (R). These three comparisons and their interaction with cultivar families (C vs. DH × CF, P vs. F_1 × CF, R × CF) were tested by error b (replications × cultivar families × lines). Duncan's multiple range test was used to test for differences between the mean values of the cultivar selfed line and the doubled-haploid selfed line within each cultivar family.
Experiment 2: The sums of squares associated with differences among the genetic entries were partitioned into differences among parents, parents vs. crosses contrast (P vs. F_1), and differences among crosses. The differences among crosses were further partitioned into sums of squares associated with general combining ability (GCA) and specific combining ability (SCA) effects. The average heterosis value for each character was calculated as the percent by which the average of the F_1's exceeds the average of the parents. The variance components of general combining ability (GCA) and specific combining ability (SCA) were computed from the expectations of the mean square values.

Results

Experiment 1: The seven cultivar families were significantly different from each other for all five characters (Table 1). In contrast to the family differences, the single degree of freedom comparisons were generally nonsignificant. The mean performance of the doubled-haploid lines was significantly different from the mean of their parental cultivars for days to flower and total alkaloids. Of the five characters examined, only leaf number had significant midparent heterosis. No reciprocal effects were indicated. Most of the interactions of the three comparisons with cultivar families were also nonsignificant. The differences between the doubled haploids and their parental cultivars were not consistent across the cultivar families for days to flower, yield, and

Table 1. Analyses of variance mean squares for the five characters evaluated in Experiment 1

Source	df	Character				
		Days to flower	Yield/plant g	Plant height cm	Leaves/plant no.	Total alkaloids %
Replications	2	14.00**	47	21.2	1.08	0.282
Cultivar Families	6	120.28**	2,200**	285.0**	52.58**	3.235**
Error a	12	5.47	496	19.2	3.58	0.098
C vs. DH	1	22.88**	424	30.3	3.09	2.015**
P vs. F_1	1	2.01	281	27.8	14.10*	0.019
R	1	7.71	92	38.1	0.00	0.003
C vs. DH × CF	6	18.81*	952**	31.7	8.69**	0.155
P vs. F_1 × CF	6	6.37	154	65.7*	3.63	0.083
R × CF	6	5.27	153	24.2	4.01	0.097
Error b	42	3.08	245	24.2	2.65	0.126

*, ** Significant difference at $\alpha = 0.05$ and $\alpha = 0.01$, respectively

Table 2. Mean performances of the cultivars, the doubled-haploid lines, and the reciprocal crosses for the five characters evaluated in Experiment 1

Character	Mean			
	Cultivar	Doubled-haploid line	Cultivar × Doubled Haploid[a]	Doubled Haploid × Cultivar[a]
Days to flower	63.8	65.3	65.3	64.4
Yield/plant, g	162.1	155.7	161.1	164.1
Plant height, cm	139.9	140.6	141.9	140.0
Leaves/plant, no.	25.1	24.5	25.6	25.6
Total alkaloids, %	2.14	1.70	1.94	1.95

[a] First parent in cross is maternal parent

Table 3. Relative performances of individual doubled-haploid lines compared to their parental cutivars for the five characters evaluated in Experiment 1

Character	Number of doubled-haploid lines		
	Significantly greater[b]	Not different	Significantly less
Days to flower	3	3	1
Yield/plant, g	0	5	2
Plant height, cm	0	7	0
Leaves/plant, no.	1	5	1
Total alkaloids, %	0	6	1

[b] Significant difference of means at $\alpha = 0.05$ in a Duncan's multiple range test for each cultivar family

leaf number. The heterosis associated with plant height also demonstrated significant interaction effects. No reciprocal effects × cultivar families interactions were evident.

The mean performances of the cultivars, the doubled-haploid lines, and the reciprocal crosses for the five characters were determined (Table 2). On the average, the doubled-haploid lines matured 1.5 days later and had 0.44% less total alkaloid content than the cultivars. The midparent heterosis for leaf number was 3.7%.

The relative performances of the individual doubled-haploid lines compared to their parental cultivars for the five characters are illustrated in Table 3. In total, 26 of the 35 comparisons were nonsignificant by Duncan's multiple range tests. All of the doubled-haploid lines were comparable to their source cultivars for plant height. In general, the performances of the doubled-haploid lines were distributed in both directions around the performances of their source cultivars. Those distributions were demonstrated in the significant cultivars vs. doubled haploids × cultivar families interactions for days to flower, yield, and leaf number.

Experiment 2: The seven doubled-haploid parental lines were significantly different for all seven characters (Table 4). The parental mean was significantly different from the F_1 mean for leaf length, leaf width, and total alkaloids. GCA was significant for all characters with

Table 4. Analyses of variance mean squares for the seven characters evaluated in Experiment 2

Source	df	Character						
		Days to flower	Yield/ plant, g	Plant height, cm	Leaf length, cm	Leaf width, cm	Leaves/ plant, no.	Total alkaloids, %
Replications	2	4.96	102	81.2	7.23	2.86	1.07	0.187
Parents	6	23.06**	1,250**	345.5**	52.54**	22.40**	23.36**	0.752**
P vs. F_1	1	0.40	634	160.0	39.36*	15.26*	0.37	0.567*
GCA	6	14.90**	825**	146.5	36.24**	23.98*	26.02**	1.481*
SCA	14	2.68	161	84.9	12.76*	3.11	2.73	0.127
Error	54	3.21	218	70.8	5.99	2.16	2.53	0.107

*, ** Significant difference at $\alpha = 0.05$ and $\alpha = 0.01$, respectively

Table 5. Mean performances of the parents and the F_1 progeny, average heterosis values, GCA variances, and SCA variances for the seven characters evaluated in Experiment 2

Character	Mean		Average heterosis value, %	Estimate of	
	Parents	F_1's		GCA variance	SCA vriance
Days to flower	66.7	66.5	−0.24	0.78**	−0.18
Yield/plant, g	154.3	160.9	4.24	40.5**	−18.9
Plant height, cm	140.0	143.2	2.28	4.09	4.72
Leaf length, cm	72.6	74.1	2.19*	1.56**	2.29*
Leaf width, cm	33.2	34.2	2.96*	1.39*	0.32
Leaves/plant, no.	24.7	24.8	0.62	1.55**	0.07
Total alkaloids, %	2.29	2.43	8.27*	0.09*	0.01

*, ** Significant difference at $\alpha = 0.05$ and $\alpha = 0.01$, respectively

the exception of plant height. Only leaf length had significant SCA variance.

The parental and F_1 means, average heterosis values, GCA variances, and SCA variances for the seven characters are given in Table 5. On the average, the hybrids had leaves 1.5 cm longer and 1.0 cm wider, and had 0.14% more total alkaloids than their parents. The average heterosis values for leaf length, leaf width, and total alkaloids were 2.19%, 2.97%, and 8.27%, respectively. GCA variance estimates were of a greater magnitude than the SCA variance estimates, indicating a predominance of additive genetic effects over dominance effects in the doubled-haploid progeny.

Discussion

The seven doubled-haploid lines did not exhibit consistent reduction of vigor when compared to their parental cultivars. The cultivar and doubled-haploid means were significantly different for two of the five characters evaluated in Experiment 1, days to flower and total alkaloids. However, the mean of the doubled-haploid lines did not differ significantly from the mean of their source cultivars for the characters that measure vigor: yield, days to flower, and leaf number. When each doubled-haploid line was compared to its cultivar source for five characters (Experiment 1), 26 of 35 comparisons were nonsignificant. In addition, average performances of doubled-haploid lines were distributed around their cultivars for days to flower, yield, and leaf number as indicated by significant C vs. DH × CF interactions for those characters. Therefore, a direct relationship between reduced vigor and doubled haploidy was not indicated for burley tobacco.

These observations are similar to those previously made with doubled haploids derived from a flue-cured tobacco hybrid (Burk and Chaplin 1980). However, the results from this study differ from the results of some previous experiments comparing doubled haploids and their source inbred cultivars (Arcia et al. 1978; Burk and Matzinger 1976). The mean of 46 doubled-haploid lines from 'Coker 139' was 9.5% lower for yield and significantly reduced for four other agronomic characters compared to the source cultivar. The doubled-haploid lines demonstrated consistent reduced vigor compared to 'Coker 139' (Burk and Matzinger 1976). In another study, doubled-haploid lines from 'Coker 139' and 'NC 95' yielded 14.6% less and were agronomically inferior to their parental cultivars (Arcia et al. 1978). Both studies concluded that reduced vigor was intrinsic to anther-derived materials from flue-cured tobacco.

Disparities between the results of this study and previous findings could be due to two factors. First, a limited sampling of genotypes could produce misleading results if reduced vigor were associated with the anther-derived materials of particular cultivars. The previously cited studies (Arcia et al. 1978; Burk and Matzinger 1976) used only two flue-cured tobacco cultivars, 'Coker 139' and 'NC 95', as the source materials. The doubled-haploid materials in this study were derived from seven different burley tobacco cultivars. The genetic differences between burley and flue-cured types could also be an important factor. For example, the TMV resistance gene from *N. glutinosa* influences the agronomic quality differently when in the two genetic backgrounds. Flue-cured lines with the resistant factor demonstrate vigor reduction and undesirable quality (Chaplin and Mann 1978; Chaplin et al. 1966) whereas burley lines with the resistant factor show no such characters (Legg et al. 1979). Second, colchicine treatment can induce mutations (De Paepe et al. 1977). The haploids in the experiments previously cited were doubled with colchicine (Arcia et al. 1978; Burk and Matzinger 1976). The doubled haploids in this study were regenerated from cells in leaf midveins that spontaneously doubled their chromosome number.

Several investigators have explained the differences between doubled-haploid lines and their source cultivars by a loss of residual heterozygosity in the inbred cultivars (Collins et al. 1973; De Paepe et al. 1977). Residual heterozygosity can also explain the differences observed in this study. According to this theory, only the inbred cultivars with heterozygous loci for a particular character could produce an anther-derived line that was significantly different for that character (a genotypic effect). Also, those cultivars could fix a gene combination in their doubled haploids for performance above or below the cultivar performance. In Experiment 1, the significant differences between the doubled-haploid lines and their parental cultivars were found only for certain genotypes and characters. In addition, the doubled-haploid lines were distributed in both directions around the performances of their source cultivars for yield, days to flower, and leaf number.

Similarities of the data from the doubled-haploid diallel (Experiment 2) and from conventional diallel (Legg et al. 1970; Matzinger et al. 1971) can also be explained with the theory of residual heterozygosity. Average heterosis values from doubled-haploid progeny were similar in magnitude to the heterosis values observed among diallel progeny from conventional inbreds. Estimates for GCA variance, and the near absence of SCA effects among doubled-haploid diallel progeny were also comparable to results from conventional materials. In doubled haploids derived from inbreds, the heterozygous loci present in cultivars would be fixed to homozygous loci. No new genes or genetic effects would be introduced by producing doubled haploids. Therefore, the genetic effects from doubled-haploid materials would be equivalent to the effects from conventional materials.

The hypothesis that differences between doubled-haploid lines and their cultivar sources are due to cytoplasmic effects was tested by comparing reciprocal crosses in Experiment 1. Since the doubled haploids and their source cultivars were each used as maternal parents, the expressions of the same nuclear genome in the cytoplasm of the cultivar and of the doubled haploid were compared. If cytoplasmic effects were responsible for reduced vigor, the cause of depression would have been eliminated in the cross with the cultivar as the female parent. Therefore, cytoplasmic effects would have been indicated by significant reciprocal effects, particularly for the two characters in which the mean of the cultivars was significantly different from the mean of the doubled haploids (days to flower and total alkaloids). However, the means of the two crosses were not different for all five characters. The nonsignificant reciprocal effects × cultivar families interactions for all characters indicated that the nonsignificant differences of the two crosses were consistent for all cultivar families.

Since most mutations are deleterious, a consistent pattern of reduced vigor in doubled-haploid materials from inbred cultivars could be an indication of an intrinsic mutagenic nature in the anther-culture process. However, the doubled-haploid lines in this study did not demonstrate consistent vigor reduction. Therefore, an intrinsic mutagenic nature of anther-culture techniques is not an adequate explanation of the differences observed between the doubled haploids and their source inbred burley cultivars in this study.

Literature

Arcia, M.A.; Wernsman, E.A.; Burk, L.G. (1978): 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

Burk, L.G.; Chaplin, J.F. (1980): Variation among anther-derived haploids from a multiple disease-resistant tobacco hybrid. Crop Sci. **20,** 334–338

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

Chaplin, J.F.; Mann, T.J. (1978): Evaluation of tobacco mosaic resistance factor transferred from burley to flue-cured tobacco. J. Hered. **69,** 175–178

Chaplin, J.F.; Matzinger, D.F.; Mann, T.J. (1966): Influence of the homozygous and heterozygous mosaic-resistance factor on quantitative characters of flue-cured tobacco. Tob. Sci. **10,** 81–84

Collins, G.B. (1977): Production and utilization of anther-derived haploids in crop plants. Crop Sci. **17,** 583–586

Collins, G.B.; Legg, P.D.; Litton, C.C. (1973): The use of anther-derived haploids in *Nicotiana.* 2. Comparison of doubled-haploid lines with lines obtained by conventional breeding methods. Tob. Sci. **18,** 40–42

Collins, G.B.; Sadasivaiah, R.S. (1972): Meiotic analysis of haploid and doubled-haploid forms of *Nicotiana otophora* and *N. tabacum.* Chromosoma **38,** 387–404

De Paepe, R.; Nitsch, C.; Godard, M.; Pernes, J. (1977): Potential from haploid and possible use in agriculture. In: Plant Tissue Culture and its Biotechnological Application (ed. Barz, W.; Reinhard, E.; Zenk, M.H.), pp. 341–352. Berlin, Heidelberg, New York: Springer

Gerstel, D.U.; Burns, J.A.; Burk, L.G. (1974): Meiosis in F_1 hybrids between haploid-derived diploids and their parents. Tob. Sci. **18,** 119–120

Gupton, C.L.; Legg, P.D.; Link, L.A.; Ross, H.F. (1974): Genotype × environment interactions in burley tobacco variety tests. Crop Sci. **14,** 811–814

Harvey, W.R.; Stahr, H.M.; Smith, W.C. (1969): Automated determination of reducing sugars and nicotine alkaloids on the same extract of tobacco leaf. Tob. Sci. **13,** 13–15

Jensen, C.J. (1974): Chromosome doubling techniques in haploids. In: Haploids in Higher Plants: Advances and potentials (ed.: Kasha, K.J.), pp. 153–190. Guelph: Univ. of Guelph

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

Legg, P.D.; Collins, G.B.; Litton, C.C. (1970): Heterosis and combining ability in diallel crosses of burley tobacco, *Nicotiana tabacum* L. Crop Sci. **10,** 705–707

Legg, P.D.; Collins, G.B.; Litton, C.C. (1979): Effects of the N mosaic-resistance factor on agronomic and chemical traits in burley tobacco. Crop Sci. **19,** 455–457

Matzinger, D.F.; Wernsman, E.A.; Ross, H.F. (1971): Diallel crosses among burley varieties of *Nicotiana tabacum* L. in the F_1 and F_2 generations. Crop Sci. **11,** 275–279

Schnell, R.J., II; Wernsman, E.A.; Burk, L.G. (1980): Efficiency of single-seed-descent vs. anther-derived dihaploid breeding methods in tobacco. Crop Sci. **20,** 619–622

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Mr. W.R. Deaton
Dr. G.B. Collins
Department of Agronomy
University of Kentucky
Lexington, Ky. 40546-0091 (USA)

Dr. P.D. Legg
Appalachian Soil and Water Conservation
Research Laboratory
P.O. Box 867, Airport Road
Beckley, W. Va. 25801 (USA)