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Comparison of Lines From Anther and Maternally-derived Dihaploids in Flue-cured Tobacco(*Nicotiana tabacum* L.)

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ABSTRACT : The present study was conducted to compare the relative efficiency of two different haploid breeding methods in tobacco varietal development. A single F_1 hybrid plant from cross of two flue-cured cultivars of *Nicotiana tabacum* L., Bright Yellow4(BY4) and NC95, was used to develop the 30 anther derived dihaploid (ADH) lines and maternally-derived dihaploid (MDH) lines utilizing *Nicotiana africana*, respectively. As compared with mid-parent, mean of ADH lines showed increase in number of leaves, delay in days to flower and narrow in width of leaf. However, no significant differences in the other investigated characters were recognized. MDH lines also showed narrow width of leaf, while no significant differences in the other characters were observed. The variations of the investigated characters were generally greater in ADH than MDH lines. MDH lines had higher height of plant and shorter days to flower than ADH lines, while the other characters did not show remarkable differences. The degree of heritability for each of the characters observed between ADH and MDH was almost the same. The characters showing high heritability value were height of plant, number of leaves, days to flower, and yield, while those showing relatively low value were length of leaf, width of leaf, and total alkaloid content. Predicted gains from selection for increased yield were calculated for both populations(ADH, MDH) and correlated responses associated with selection for yield were estimated. Height of plant, width of leaf, days to flower, and concentration of reduceing sugar would be expected to improve with selection for yield much faster in the MDH population than in the ADH.

Key words : ***Nicotiana africana*, ADH(anther derived dihaploid), MDH(maternally-derived dihaploid), haploid breeding method**

INTRODUCTION

Plant breeders have been acutely interested in haploid techniques which allow segregating materials to be rapidly advanced to complete homozygosity in a single generation. In tobacco, *Nicotiana tabacum* L., the ease of haploid production *via* anther-culture(Bourgin and Nitsch, 1967; Nakata and Tanaka, 1968; Nitsch and Nitsch, 1969) has led to many investigations of this procedure as related to plant improvement.

The use of a haploid-dihaploid breeding approach *via* anther-culture could result in a three to four-year time savings in the inbreeding or genotype stabilization process. This procedure would have no affect on the testing requirement of

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cultivar development. Haploid lines produced by anther-culture and chromosome doubled with colchicine (Burk *et al.*, 1972) or by midrib culture (Kasperbauer and Collins, 1972) have been found to be genetically stable(Collins *et al.*, 1974) and free of detectable chromosomal aberrations (Gerstel *et al.*, 1974).

Variability and reduced vigor among anther-derived dihaploid families from inbred parents have been reported by several investigators (Burk and Matzinger, 1976; Collins *et al.*, 1973; Legg and Collins, 1968; Nakamura *et al.*, 1974; Oinuma and Yoshida, 1974). This variation has been attributed to several consequences of the haploid-diploid anther-culture procedure. Residual heterozygosity in the parental cultivars has been proposed by Burk and Matzinger(1976) and Collins *et al.* (1973) to be a cause of reduced vigor of the dihaploid lines. However, the work by Arcia *et al.* (1978) with F_1 and F_2 generations of dihaploids and conventionally derived F_1 and F_2 generations does not support this hypothesis. The potential mutagenic effects of colchicine have also been suggested as a reason for reduced vigor in dihaploid lines. Burk and Matzinger (1976) found that dihapolid lines produced by colchicine doubling were not significantly different from dihaploid lines that had doubled spontaneously, and in both instances the dihaploid lines were reduced in vigor and productivity when compared to conventionally derived materials from the same origin(Brown *et al.*, 1983; Wernsman *et al.*, 1984).

Maternal haploids can be obtained by pollination of plants of *N. tabacum* with *N. africana*(Burk *et al.*, 1979). Numerous seeds develop in fruits from this cross, but germinating interspecific hybrid seedlings are highly lethal (99.9%). Surviving F_1 plants consist of mixtures of aneuploid interspecific hybrids and maternal haploids. The chromosomes of the maternal haploids are derived from the *N. tabacum* female plant. The procedure is very simple, but requires technical skill to distinguish phenotypically the aneuploid interspecific hybrids from maternal haploids in seedling stages. Chromosome-doubled haploids obtained by this technique are superior to ADH lines from the same parental sources and more closely resemble the performance of conventionally developed inbred genotypes(Wernsman *et al.*, 1984; Yung and Wernsman, 1990).

Using this method, a new cultivar, KF113 (Chung *et al.*, 1994), have been released to growers, and a number of experimental entries have been entered in official testing programs in Korea. The objetive of our study was to compare the lines between anther and matermally-derived dihaploids in flue-cured tobacco.

MATERIALS AND METHODS

A Single hybrid plant from a cross of two flue-cured tobacco cultivars of Bright Yellow 4 (designated BY4) and NC95, was used to develop the 30 anther derived dihaploid(ADH) lines and maternally derived dihaploid(MDH) lines utilizing *Nicotiana africana*, respectively. Haploids sporophytes were developed by cultured anthers (Nakamura *et al.*, 1974) and *N. africana* method (Burk *et al.*, 1979), and the haploid breeding process are shown in Fig. 1.

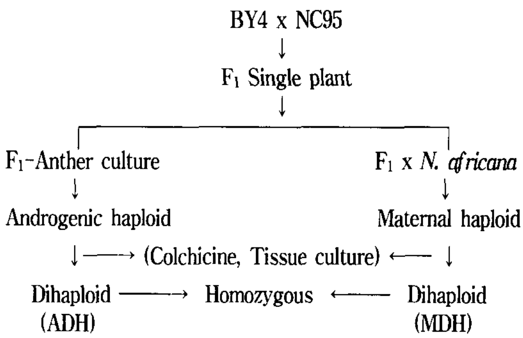

Fig. 1. Diagram of developing the dihaploids from F_1 single plant of BY4 x NC95 by anther culture and *N. africana* method.

The complementary chromosome of the haploids was doubled using the colchicine method (Kumashiro and Oka, 1978) in anther culture. The maternal haploids were chromosome-doubled using tissue culture(Kasperbauer and Collins, 1972).

The tests were conducted at Suwon Experiment Station of the Korea Ginseng & Tobacco Research Institute using the randomized block design with 3 replication in 1990. Cultural practices including fertilization, cultivation, sucker and pest control were those normally recommended for flue-cured tobacco production at this research station. Entries were grown in single row, 20 competitive plant plots with spacings of 45cm between plants and 110cm between rows.

Data were collected on yield of curred leaf, days to flower, plant height, leaves per plant, length of leaf and width of largest leaf, concentrations of reducing sugar (Harvey and Palmer, 1971) and total alkaloids (Cundiff and Markunas, 1964) in the cured leaf. Heritabilities were estimated and expected genetic advances from selection for increased yields in the two populations were estimated ($h= \sigma^2/\sigma^2G+\sigma^2E$). Genetic gains (Frey and Horner, 1955) were estimated using analysis of products between pairs of variables and individual analysis of variance for each variable.

RESULTS AND DISSCUSSION

Distributions for mean yield the anther derived dihaploid(ADH) and maternally derived dihaploid (MDH) populations are shown in Figure 2. Within plots, dihaploid lines were highly uniform but the dihaploid population was more variable, and ADH mean yield of leaf were reduced 2.9% compared to the MDH population. Phenotypic variances were greater in the ADHs for yield, leaves per plant, days to flower, total alkaloids, and reducing sugar, while phenotypic variances were greater in the MDH population for plant height, length and width of leaf (Table 1).

Significant genetic variation among lines was observed for yield within both in the ADH and MDH populations, indicating that yields could be improved by selection in both populations. Two families in the ADH population performed higher than NC95 check, and 16 families yields of leaf exceeded that of Bright Yellow 4 (Fig. 2). The ADH families yideded 1.5% higher than BY4 and 12.5% less than NC95 did . Four families among MDH lines yielded greater than NC95, while 20 out-performed than BY4. Both populations exhibited similar growing characteristics to BY4 and NC95 (Table 2). Sugar contents of cured leaf of the two populations were similar and were not significantly different from the parental cultivars. Significant variability was observed among AHD and MDH populations. Cured leaf of the ADHs possessed lower total alkaloids than MDHs, but

Table 1. Partitioning of mean squares for agronomic characters and chemical constituents between the breeding methods

	Height of plant	Leaves per plant	Length of leaf	Width of leaf	Days to flower	Yield	Total alkaloids	Reducing sugar
Within Family								
ADH[1)]	707.897**	5.235**	17.306**	8.380**	6.008**	864.336**	0.382**	22.854**
MDH[2)]	806.815**	6.280**	15.010**	7.300**	13.111**	997.103**	0.293**	36.127**
Between Families								
Parent vs. ADH	119.205	2.393	12.939	25.573**	14.601**	541.451	0.464	0.343
Parent vs. MDH	24.025	6.292	0.591	17.336**	0.851	92.010	0.0007	0.931
ADH vs. MDH	288.800	8.107	63.963	6.385	67.222*	1496.448	0.745	19.208

* $P<0.05$, ** $P<0.01$.

1) ADH : F_1-Anther derived doubled haploid lines.

2) MDH : F_1-Maternally derived doubled haploid lines.

Table 2. Mean and range for characters of anther-derived doubled haploid(ADH) lines and maternally-derived doubled haploid(MDH) lines in a single F_1 hybrid plant from cross BY4 and NC95

Character	Parents		Mid-Parent	Doubled haploid			
				ADH		MDH	
	BY4	NC95		Mean±s	Range	Mean±s	Range
Height of plant, cm	156	131	143.5	142.7±2.8	97-169	145.3±2.9	123-191
Leaves per plant, no.	19.5	20.7	20.1	20.8±0.2	15.3-22.3	20.4±0.3	15.9-23.0
Length of leaf, cm	45.4	47.0	46.2	45.0±0.4	39.0-49.0	46.2±0.4	41.8-51.5
Width of leaf, cm	24.5	26.8	25.7	24.0±0.3	17.2-26.7	24.2±0.3	21.1-27.3
Days to flower	61	63	62	64.1±0.2	61-68	62.8±0.4	54-66
Yield, Kg/10a	191	218	204.5	193.8±3.1	135-223	199.6±3.3	158-232
Total alkaloids, %	3.17	2.46	2.82	2.7±0.06	1.68-3.69	2.38±0.05	2.13-20.5
Reducing sugar, %	15.6	18.3	17.0	15.3±0.05	10.5-20.2	16.0±0.5	10.9-20.5

was not significantly different from the mean of the ADH and MDH populations.

Table 2 showed the performance of dihaploid progenies of the ADH and MDH methods. Growth of the ADH lines was generally depressed compared with that of parents (BY4 and NC95). The ADH lines flowered later, had greater leaves, and were 12.5 percent lower in yield than NC95. None of the ADH lines was significantly larger than parents in the length of leaf and width of leaf.

For days to flower, 23 of the 30 ADH lines flowered significantly later than parents. Over 50 percent of the ADH lines did not differ significantly in leaves per plant, length of leaf, width of leaf, yield, total alkaloids, and reducing sugar as compared to parents (Table 3).

MDH lines were also similar in all characters. Most of the characters did not differ significantly as compare to parents. None of the MDH lines was significantly greater than parents in width of leaf, total alkaloids, and reducing sugar. Among the selection response from selecting the highest yielding 30% of the ADH and MDH lines, cured

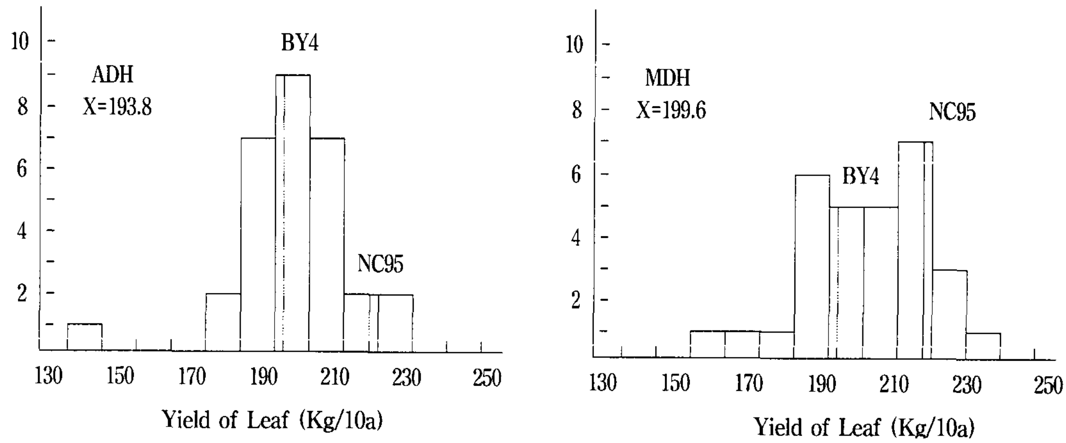

Fig. 2. Frequency of distribution of mean yield of anther-derived dihaploid (ADH) and maternally-derived dihaploid (MDH) lines

Table 3. Number of anther-derived doubled haploid(ADH) and maternally-derived doubled haploid(MDH) lines from cross BY4 x NC95 showing the greater than, no difference from and less than the midparent value for certain characters.

	Height of plant	Leaves per plant	Length of leaf	Width of leaf	Days to flower	Yield	Total alkaloids	Reducing sugar
ADH								
Greater*	4	11	0	0	23	1	1	4
Not different	14	17	24	22	6	20	25	20
Less*	12	2	6	8	1	9	4	6
MDH								
Greater*	5	9	1	0	12	4	0	0
Not different	16	18	28	19	13	17	29	30
Less*	9	3	1	11	5	9	1	0

* Significantly different at the 0.05 level of probability.

Table 4. Selection response from selecting the highest yielding 30% of the lines in anther-derived doubled haploid(ADH) and maternally-derived doubled haploid (MDH) from a BY4 x NC95 cross

	Yield	Heitht of plant	Leaves per plant	Days to flower	Total alkaloids	Reducing sugar
ADH						
Population X	193.8	142.7	20.8	64.1	2.70	15.3
Best 9 lines (based on yield)	211.1	147.8	21.3	64.4	2.77	15.7
Heritability	0.62	0.80	0.71	0.65	0.29	0.65
Selection response*	10.73	4.08	0.36	0.26	0.02	0.26
MDH						
Population X	199.6	145.3	20.3	62.8	2.83	16.0
Best 9 lines (based on yield)	219.7	160.4	20.8	63.6	2.98	15.5
Heritability	0.78	0.83	0.77	0.81	0.22	0.15
Selection response*	15.68	12.53	0.31	0.65	0.03	-0.08
Check						
BY4	191.0	156.0	19.5	61.0	3.17	15.6
NC95	218.0	131.0	20.7	63.0	2.46	18.3

* : Predicted genetic gains and correlated responses

leaf of the dihaploid lines possessed similar total alkaloids between the mean of the ADH and MDH populations.

Heritabilities were computed for agronomic and chemical constituents in the two populations and were found to be higher in height of plant, leaves per plant and days to flower. The selection responses differed in the two populations for height of plant, days to flower, and reducing sugar. Height of plant, and days to flower would be expected to improve with selection for yield much faster in the MDH population than in the ADH. Reducing sugar was predicted to decrease in the MDH by-0.08% for yield selection but would be expected to remain unchanged in the ADH (Table 4).

Both ADH and MDH lines were agronomically inferior to selfed progenies of the parental plants for many parameters, but the degree of inferiority were very similar for the two types of doubled haploids. Yield of cured leaf of the ADH lines were only 95% of the yields of the mid parents, while the MDH lines yielded 98% of that of the parents. The ADH lines were significantly different from the MDH lines from the same parental plant for several traits. ADH lines were slightly lower in yield, shorter in height of plant, later in days to flower, smaller in leaf length and leaf width, but not in the number of leaves. ADH lines had lower concentrations of total alkaloids and reducing sugar. Genetic variability among ADH lines was greater in several characters than that among MDH lines from the same sources.

These data are interpreted as indicating that the genetic nature of the vegetative nucleus of microspores from which the haploid plantlets arise during anther culture (Kasperbauer *et al.*, 1983). This is different from the embryo sac nucleus providing the maternal haploids. These results suggest that the magnitude of genetic variability induced by the *N. africana* genetic system of maternal haploid production is not large. Although MDH lines are slightly lower yielding than inbred cultivars from which they have been derived, differences are small. Consequently the production of gynogenic haploids *via* the *N. africana* procedure and their subsequent chromosome doubling results in competitive homozygous genotypes.

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