1—17 (1922). — 14. ROBINSON, H. F., R. E. COMSTOCK, and P. H. HARVEY: Estimates of heritability and the degree of dominance in corn. Agron. J. **41**, 353—359 (1949). — 15. ROBINSON, H. F., R. E. COMSTOCK, and P. H. HARVEY: Genotypic and phenotypic correlations in corn and their implications in selection. Agron. J. **43**, 282—287 (1951). — 16. ROBINSON, H. F., R. E. COMSTOCK and P. H. HARVEY: Genetic variances in open-pollinated varieties of corn. Genetics **40**, 45—60 (1955). — 17. SMITH, L. H.: The effect of selection upon certain physical characters of the corn plant. Ill. Agr. Exp. Sta. Bul. 132 (1909). — 18. SPRAGUE, G. F.: Corn and corn improvement. New York, N. Y.: Academic Press, Inc., 1955. — 19. STUBER, C. W., R. H. MOLL and W. D. HANSON: Genetic variances and interrelationships of six traits in a hybrid population of corn (*Zea mays* L.). Crop Science **6**, 455—458 (1966). — 20. WILLIAMS, J. C., L. H. PENNY and G. F. SPRAGUE: Full-sib and half-sib estimates of genetic variance in an open-pollinated variety of corn (*Zea mays* L.). Crop Science **5**, 125—129 (1965).

Genetic Diversity and Heterosis in *Nicotiana* *

I. Interspecific Crosses [1]

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Summary. Two flue-cured varieties of *N. tabacum* were crossed to putative progenitor species and to distantly related species. Heterosis for yield, plant height, and number of leaves was largest for crosses to progenitor species, particularly to *N. otophora* and *N. tomentosiformis*. The magnitude of this heterosis appeared to be greater than estimates presented in the literature for crosses among varieties of *N. tabacum*. An additional study presented some evidence for the genomic basis of heterosis in crosses of *N. tabacum* with *N. tomentosiformis* and *N. sylvestris*.

Introduction

A number of studies have been conducted in *Nicotiana* to obtain estimates of heterosis in F_1 hybrids. This heterosis, when measured as general vigor of the plant, is similar to luxuriance described by DOBZHANSKY (1952). Throughout this paper, the point of reference will be the mean of both parents, and heterosis will pertain to the deviation of F_1 from mid-parent.

Early reports on results of hybridization in *N. tabacum* L. pointed out the lack of heterosis in the varieties and strains which existed at that time. DARWIN (1876) made special note of crosses among varieties of *N. tabacum* by describing the lack of vigor in crosses as a curious case. SETCHELL *et al.* (1922) summarized results of intervarietal crosses in *N. tabacum* as follows: "When we are dealing with complex differences, the F_1 is commonly intermediate in character expression between the two parents. Not only is this true as respects the F_1 plant as a whole but it is also true for individual characters".

In recent years there has been a large amount of data on heterosis presented with a wide variety of strains of *N. tabacum*. Examples of these studies are given by RAVE (1934), OKA *et al.* (1959), MURTY *et al.* (1962), POVILAITIS (1964), OKA and EGUCHI (1965), MURTY (1965), CHAPLIN (1966), MARANI and SACHS (1966), MATZINGER *et al.* (1960), MATZINGER and MANN (1962), MATZINGER *et al.* (1962), MANN *et al.* (1962), and AYCOCK *et al.* (1963). In general, heterosis values for various characters ranged from no heterosis to only modest amounts. However, in a few cases the F_1 hybrid exceeded both parents. In many of these studies, increased heterosis was attributed to increased genetic diversity, although many crosses of varieties with apparently diverse origin did not exhibit heterosis.

Interspecific hybridization within *Nicotiana* has been practiced extensively, however, there is little information on comparisons of the interspecific hybrids with the parent species for growth characteristics in replicated trials. Results of early observations on differences among crosses in interspecific hybridization are summarized by KOSTOFF (1941 to 1943). When crosses were made between *N. tabacum* and other species, the crosses to *N. sylvestris* Speg. and Comes and *N. rustica* L. were very vigorous, whereas the crosses to more distantly related '*N. sanderae*' or *N. alata* Link and Otto produced dwarf hybrids. A large number of flue-cured varieties of *N. tabacum* were compared with their crosses to *N. sylvestris* by MANN and WEYBREW (1958). Although *N. sylvestris* was not included in the test, the relative performance of the hybrids compared with the flue-cured parents indicated that appreciable heterosis was being exhibited in the crosses. ASHTON (1946) summarized reports of heterosis in many of the self-pollinated crops, including tobacco. His survey indicated some evidence that greater heterosis was exhibited from interspecific crosses than intraspecific crosses.

The present study was designed to measure the heterotic response when flue-cured varieties of *N. tabacum* were crossed to species of *Nicotiana* with varying degrees of phylogenetic diversity. *N. tabacum* ($n = 24$) is an amphidiploid of two 12-chromosome species. The probable progenitors are *N. sylvestris* (genome designation, S′S′) and a member of the Tomentosae section, either *N. otophora* Grisebach, favored by GOODSPEED (1954), or *N. tomentosiformis* Goodspeed favored by GERSTEL (1960). The genomic designation of the Tomentosae section representative is T′T′. The species representatives in the crosses were these three candidates as progenitors of *N. tabacum*, and two more distantly related diploid species, *N. glauca* Graham and *N. glutinosa* L. In addition, three varieties of *N. tabacum* were crossed to KOSTOFF's amphiploid 4X (*N. sylvestris* × *N.*

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tomentosiformis) to obtain additional information on genomic contribution to heterosis.

Materials and Methods

Two flue-cured varieties of *N. tabacum*, SC58 and Coker 139, were each crossed with single accessions of *N. otophora* v. La Quinta (UCBG 37-23[2]), *N. tomentosiformis* (UCBG 59-G-54), *N. sylvestris* v. Catamarca (UCBG 49-G-87), and *N. glutinosa* (Kehr[3]) and two accessions of *N. glauca*, a yellow flowered strain (Kehr[3]) and v. *lateritia*, a purple flowered strain (UCBG 37-12).

Each species representative and the F_1 hybrids were seeded individually in paper cups in the greenhouse and transplanted to the field when the *N. tabacum* entries were approximately six inches tall. The material was evaluated in a randomized complete block design with three replications and 20 plants per plot at Clayton, North Carolina, in 1964. Cultural practices were those normally employed for flue-cured tobacco.

Plants were topped at a point two leaves below the first floral branch of the inflorescence as they flowered. Days to flower were recorded as the number of days from transplanting to flower of 50 percent of the plants in the plot. The number of leaves and plant height were recorded on ten competitive plants per plot and converted to plot means. As leaves of the flue-cured varieties matured they were removed from the plants at weekly intervals and cured. Although normal ripening did not occur in the species other than *N. tabacum* or in the hybrids, leaves were removed from stalk positions of the entries corresponding to the time of ripening in the flue-cured varieties. A simulated cure was obtained for these entries by placing them in the curing barn with the flue-cured varieties. At the end of the season the leaves from each plot were weighed and converted to a yield per acre basis.

In a separate study, three varieties of *N. tabacum*, *viz.* Hicks Broadleaf, NC 95, and Coker 316, were each crossed to KOSTOFF's amphiploid 4X (*N. sylvestris* × *N. tomentosiformis*). This amphiploid was originally received by R.E. CLAUSEN from D. KOSTOFF and it has been maintained for several decades by selfing. The three *N. tabacum* varieties, KOSTOFF's amphiploid, and the three F_1 hybrids were evaluated in four replications at Whiteville, North Carolina, in 1966. The procedures of field experimentation were the same as those of the previous study.

Results

The means for the species and interspecific hybrids are presented in Table 1. *N. otophora*, *N. tomentosiformis*, and the two selections of *N. glauca* had not flowered at the completion of harvest of all of the leaves 105 days after transplanting the experiment to the field. Differences among the species were significant ($P = .01$) for all characters. The extreme range of performance of the species is indicated by a fifteen-fold difference in yield, an eight-fold difference in final plant height, a five-fold difference in number of leaves, and a six-fold difference in days to flower.

Table 1. *Performance of Nicotiana species and interspecific hybrids.*

Entries	Yield lbs./A.	Height cm.	Leaves no.	Flower days
N. tabacum (C139)	3384	132	30	71
N. tabacum (SC58)	2318	123	23	56
N. otophora	1800	105	27	105[a]
N. tomentosiformis	1613	171	42	105[a]
N. sylvestris	1555	69	20	51
N. glauca (y)	690	220	64	105[a]
N. glauca (p)	490	243	60	105[a]
N. glutinosa	230	35	12	18
C139 × *N. otophora*	3781[b]	216[b]	34	78
SC58 × *N. otophora*	2898[b]	190[b]	28	74
C139 × *N. tomentosiformis*	3129	225[b]	32	79
SC58 × *N. tomentosiformis*	2731[b]	241[b]	35	77
C139 × *N. sylvestris*	2885	120	21	55
SC58 × *N. sylvestris*	2026	112	18	47
C139 × *N. glauca* (y)	2014	201	41	68
SC58 × *N. glauca* (y)	1364	193	40	63
C139 × *N. glauca* (p)	1786	202	37	67
SC58 × *N. glauca* (p)	1232	164	29	57
C139 × *N. glutinosa*	1536	65	12	36
SC58 × *N. glutinosa*	1186	76	11	36
LSD .05	260.0	20.5	4.5	4.9
.01	350.1	27.5	6.0	6.6

[a] Plants had not flowered at termination of experiment 105 days following transplanting.
[b] Hybrid performance exceeds high parent at $P = .01$.

Differences among the hybrids were also significant ($P = .01$) for all characters. The 12 hybrids were arranged in a 2 × 6 table, with the two varieties of *N. tabacum* against the other six species. Estimates of sources of variation were obtained for differences between the two *N. tabacum* varieties measured in interspecific hybrids (1 df), differences among the species other than *N. tabacum* measured in interspecific hybrids with the two varieties of *N. tabacum* (5 df), and interaction between *N. tabacum* parents and the remaining species parents (5 df).

Average performance of the two *N. tabacum* varieties in the interspecific hybrids was significantly different for all characters (Table 2) with the performance of the hybrids in the same direction as the *N. tabacum* varieties *per se* (Table 1). However, the differences were reduced in the hybrids for yield, number of leaves, and days to flower, whereas the 9 cm. difference of the two varieties for plant height was maintained in the hybrids.

Differences among the six other species measured in F_1 hybrids with *N. tabacum* were also significant for all characters. There was some interaction of *N. tabacum* by other species parents in hybrids. In the *N. tabacum* × *N. tomentosiformis* hybrid, plant height and number of leaves were less in the C 139 cross than in the SC 58 cross, and in the *N. tabacum* × *N. glutinosa* hybrid, plant height was less in the C 139 cross than in the SC 58 cross. In all other cases the

Table 2. *Performance of the two N. tabacum varieties averaged in all interspecific hybrids.*

Entries	Yield lbs./A.	Height cm.	Leaves no.	Flower days
C139 Hybrids	2522	172	30	64
SC58 Hybrids	1906	162	27	59
Difference	616**	10*	3**	5**

* Difference significant at $P = .05$.
** Difference significant at $P = .01$.

[2] University of California Botanical Garden accession number.
[3] Seed received from A. E. KEHR in 1949.

Table 3. *Percent heterosis of F_1 interspecific hybrids above mid-parent average.*

Entries	Yield lbs./A.	Height cm.	Leaves no.	Flower days
C139 × *N. otophora*	46**	82**	18**	-11**
SC58 × *N. otophora*	41**	66**	11	- 8**
C139 × *N. tomentosiformis*	25**	48**	-11*	-10**
SC58 × *N. tomentosiformis*	39**	64**	8	- 4
C139 × *N. sylvestris*	17**	19*	-16*	-10**
SC58 × *N. sylvestris*	5	16	-18*	-13**
C139 × *N. glauca* (y)	- 1	14**	-12**	-23**
SC58 × *N. glauca* (y)	- 9	12*	- 8	-21**
C139 × *N. glauca* (p)	- 8	8	-17**	-24**
SC58 × *N. glauca* (p)	-12	-10*	-30**	-29**
C139 × *N. glutinosa*	-15*	-22*	-44**	-18**
SC58 × *N. glutinosa*	- 7	- 4	-36**	- 3

* F_1 deviates from midparent at $P = .05$.
** F_1 deviates from midparent at $P = .01$.

relative direction of performance of the hybrids was the same as for the *N. tabacum* varieties *per se.*

Hybrids of SC 58 and C 139 with *N. otophora* and *N. tomentosiformis* were taller than the taller parent of the cross and except for C 139 × *N. tomentosiformis* their yield exceeded the high yielding parent (Table 1). The percent heterosis above mid-parent for all hybrids is presented in Table 3. For yield and plant height, some hybrids were above mid-parent and some were below. For number of leaves, there was a reduction in leaf number for all hybrids except for the crosses to *N. otophora* and SC 58 × *N. tomentosiformis*. All of the hybrids flowered earlier than the mid-parent. Although not all crosses exhibited heterosis, there was a definite trend for a reduction in heterosis for yield, plant height and number of leaves as one goes from the top to the bottom of Table 3.

The 1966 results from crossing three varieties of *N. tabacum* to KOSTOFF's amphiploid are presented in Table 4. The amphiploid yielded only one-fourth of the *N. tabacum* varieties, was shorter, had fewer leaves, and flowered earlier. Hybrids between the amphiploid and the *N. tabacum* varieties had yields not different from the mid-parents, were taller than the tallest parents, and flowered similar to the early flowering amphiploid. Leaf number of the hybrids was below mid-parent except for the Hicks cross which had the same leaf number as the Hicks paıent.

Table 4. *Comparative performance of N. tabacum varieties,* KOSTOFF's *amphiploid and hybrids between N. tabacum and the amphiploid.*

Entries	Yield lbs./A.	Height cm.	Leaves no.	Flower days
4X (*N. syl.* × *N. tom.*)	537	108	12	49
Hicks	1832	122	14	56
NC95	2154	127	18	60
C316	2187	138	17	64
Hicks × 4X (*N. syl.* × *N. tom.*)	1168	140	14	50
NC95 × 4X (*N. syl.* × *N. tom.*)	1288	142	13	51
C316 × 4X (*N. syl.* × *N. tom.*)	1388	148	13	56
LSD .05	183.1	6.6	2.2	2.0
.01	244.2	8.8	3.0	2.7

Discussion

Since all crosses in the first study were between diploid and amphidiploid forms, the hybrids were triploid. This led to comparisons among different levels of ploidy; however, all heterosis comparisons between hybrids were based on comparable levels. If the genomic designation of *N. tabacum* is SSTT (absence of primes denotes the evolved form of the progenitor genomes), and the genome of the species to which *N. tabacum* was crossed is designated X, then

$$\% \text{ heterosis} = \frac{STX - \frac{SSTT + XX}{2}}{\frac{SSTT + XX}{2}} \times 100 .$$

Thus, the crosses of *N. tabacum* to progenitor species lead to vigor in excess of the high parent, whereas in more diverse crosses the hybrids may be no better than the less vigorous parent in the cross.

From this study, no information is available on the absolute magnitude of heterosis independent of level of ploidy. If the triploid level is the optimum level of polyploidy the heterosis values may be inflated.

By incorporating results from the second study, some information can be obtained on the joint effects of polyploidy and genomic constitution for *N. tabacum* and the proposed progenitors, *N. sylvestris* and *N. tomentosiformis*. Although the results from the two experiments were obtained in different years, and with different varieties of *N. tabacum*, an attempt has been made to combine general results on heterosis through the *N. tabacum* included in each experiment. In Table 5 the rank of the genomes is presented for *N. tabacum* ($SSTT$), KOSTOFF's amphiploid ($S'S'T'T'$), the hybrid between them ($SS'TT'$), and crosses of *N. tabacum* with *N. sylvestris* ($SS'T$) and with *N. tomentosiformis* (STT').

Table 5. *Rank of various combinations of N. tabacum with progenitor genomes.*

Rank	Characters: Yield	Height	Leaves	Flower
1	STT′	STT′	STT′	STT′
2	SSTT	SS′TT′	SSTT	SSTT
3	SS′T	SSTT	SS′TT′	SS′TT′
4	SS′TT′	SS′T	SS′T	S′S′T′T′
5	S′S′T′T′	S′S′T′T′	S′S′T′T′	SS′T

There does not seem to be a general association of increased heterosis with triploidy since, on the average, the two triploids rank first and fourth. An interesting phenomenon then is the fact that *N. tabacum* when crossed to *N. tomentosiformıs* exhibits such large heterosis, whereas the cross of *N. tabacum* to KOSTOFF's amphiploid exhibits essentially none except for plant height. It would appear that the combination of T from *N. tabacum* and T' from *N. tomentosiformis* exhibits increased vigor when in the presence of S from *N. tabacum*, but when the S' from *N. sylvestris* is added there is a decrease in performance. The genome, S', from *N. sylvestris* does not seem to contribute as much to the heterotic effect since, with one exception, the three combinations which contain an S' are at the bottom of Table 5 for all characters, independent of level of polyploidy. The somewhat reduced heterotic response when crossing *N. tabacum* to *N. sylvestris* may indicate that *N. sylvestris* is beyond the optimal diversity, since GERSTEL (1963) found that some chromosomes of *N. sylvestris* are more differentiated from *N. tabacum* than are those of any of the species of the Tomentosae section (GERSTEL 1960).

The phenomenon of maximum heterosis associated with an optimal level of genetic diversity agrees with other reports in the literature. MOLL *et al.* (1965) compared hybrids of populations of maize obtained from various geographical regions. When the hybrids were ranked according to ancestral relationships, heterosis increased with increased divergence to a certain level, then decreased as the relationship became more diverse. T. MUKAI (personal communication) related heterosis to the number of heterozygous loci arising from spontaneous mutations in *Drosophila*. With a few loci heterozygous, the heterozygote was superior to both homozygotes, but as more and more loci were made heterozygous this heterotic effect decreased.

The relationship of genetic diversity to heterosis in this paper was limited to morphological characters only. Since all of these interspecific hybrids are sterile, one could not have extended the crosses over such a wide range if seed characters were measured. In other organisms, where heterosis measurements are related to reproductive traits, sterile crosses are beyond the optimal level of diversity. Sterility of the F_1 hybrids is not thought to have affected the morphological characters since flower heads were removed from all plants prior to seed formation.

The failure of some of the species to flower is due to qualitative genes for photoperiodic response. The relationships in this paper relate to cultural practices of *N. tabacum* and to long days. Somewhat different relationships would probably be obtained if the experiment was conducted with short days and with different cultural practices.

From the data in this paper compared to intraspecific crosses of *N. tabacum*, it would appear that the optimum level of heterosis in crosses involving *N. tabacum* may be at or near the progenitor species level, particularly the representative of the Tomentosae section. In view of the pronounced heterotic effect from the probable progenitor genomes, a procedure has been presented by WERNSMAN and MATZINGER (1966) to incorporate this desired vigor into *N. tabacum*. Whether *N. tabacum* has merely lost genes with additive effects for these characters or whether the vigor loss has resulted from loss of dominance by self-fertilization is not known, but further studies should help answer this question. This study did not point out any complex interactions involving the *N. tabacum* varieties C 139 and SC 58 since their relative performance in hybrids (Table 2) was similar to their relative performance *per se*.

Zusammenfassung

Zwei für Röhrentrocknung geeignete Sorten von *N. tabacum* wurden mit vermutlichen Ausgangs- und mit entfernt verwandten Arten gekreuzt. Die Heterosis für Ertrag, Pflanzenhöhe und Blattanzahl war am stärksten bei Kreuzungen mit den Ausgangsarten, besonders mit *N. otophora* und *N. tomentosiformis*. Das Ausmaß dieser Heterosis schien die in der Literatur berichteten Schätzungen für Kreuzungen zwischen Sorten von *N. tabacum* zu übertreffen. Eine weitere Untersuchung erbrachte Hinweise für die genomatische Grundlage der Heterosis bei Kreuzungen von *N. tabacum* mit *N. tomentosiformis* und *N. sylvestris*.

Literature

1. ASHTON, T.: The use of heterosis in the production of agricultural and horticultural crops. Imp. Bur. of Plant Breeding and Genetics, School of Agriculture, Cambridge, England (1946). — 2. AYCOCK, M. K., Jr., T. J. MANN and D. F. MATZINGER: Investigations with a form of cytoplasmic male-sterility in flue-cured tobacco. Tobacco Sci. **7**, 130—135 (1963). — 3. CHAPLIN, J. F.: Comparative performance of F_1 flue-cured tobacco hybrids and their parents. I. Agronomic and quality characteristics. Tobacco Sci. **10**, 126—130 (1966). — 4. DARWIN, C.: The effects of cross and self-fertilization in the vegetable kingdom. London 1876. — 5. DOBZHANSKY, TH.: Nature and origin of heterosis. Heterosis, pp. 218—223. Ames: Iowa State College Press 1952. — 6. GERSTEL, D. U.: Segregation in new allopolyploids of *Nicotiana*. I. Comparison of 6X (*N. tabacum* × *tomentosiformis*) and 6X (*N. tabacum* × *otophora*). Genetics **45**, 1723—1734 (1960). — 7. GERSTEL, D. U.: Segregation in new allopolyploids of *Nicotiana*. II. Discordant ratios from individual loci in 6X (*N. tabacum* × *N. sylvestris*). Genetics **48**, 677—689 (1963). — 8. GOODSPEED, T. H.: The Genus *Nicotiana*. Waltham: Chronica Botanica 1954. — 9. KOSTOFF, D.: Cytogenetics of the genus *Nicotiana*. Karyosystematics, genetics, cytology, cytogenetics, and phylesis of tobaccos. Sofia: State Printing House 1941—1943. — 10. MANN, T. J., G. L. JONES and D. F. MATZINGER: The use of cytoplasmic male sterility in flue-cured tobacco hybrids. Crop Sci. **2**, 407—410 (1962). — 11. MANN, T. J., and J. A. WEYBREW: Manifestations of hybrid vigor in crosses between flue-cured varieties of *N. tabacum* and *N. sylvestris*. Tobacco Sci. **2**, 120—125 (1958). — 12. MARANI, A., and Y. SACHS: Heterosis and combining ability in a diallel cross among nine varieties of oriental tobacco. Crop Sci. **6**, 19—22 (1966). — 13. MATZINGER, D. F., and T. J. MANN: Hybrids among flue-cured varieties of *Nicotiana tabacum* in the F_1 and F_2 generations. Tobacco Sci. **6**, 125—132 (1962). — 14. MATZINGER, D. F., T. J. MANN and C. C. COCKERHAM: Diallel crosses in *Nicotiana tabacum*. Crop Sci. **2**, 383—386 (1962). — 15. MATZINGER, D. F., T. J. MANN and H. F. ROBINSON: Genetic variability in flue-cured varieties of *Nicotiana tabacum*. I. Hicks Broadleaf × Coker 139. Agron. J. **52**, 8—11 (1960). — 16. MOLL, R. H., J. H. LONNQUIST, J. V. FORTUNO and E. C. JOHNSON: The relationship of heterosis and genetic divergence in maize. Genetics **52**, 139—144 (1965). — 17. MURTY, B. R.: Heterosis and combining ability in relation to genetic divergence in flue-cured tobacco. Ind. J. of Genet. and Plant Breeding **25**, 46—56 (1965). — 18. MURTY, B. R., G. S. MURTY and M. V. PAVATE: Studies on quantitative inheritance in *Nicotiana tabacum* L. II. Components of genetic variation for flowering time, leaf number, grade performance and leaf burn. Der Züchter **32**, 361—369 (1962). — 19. OKA, M., and K. EGUCHI: Inheritance of yields and alkaloid content in flue-cured tobacco. Jap. J. Breeding **15**, 47—52 (1965). — 20. OKA, M., T. TOKITSU and Y. MURAOKA: Inheritance of some characters in the crosses of tobacco races and genetic gain of the selected strains. Okayama Tob. Exp. Sta. Bull. **17**, 87—93 (1959). — 21. POVILAITIS, B.: Inheritance of certain quantitative characters in tobacco. Can. J. Genet. Cytol. **6**, 472-479 (1964). — 22. RAVE, L.: Heterosis beim Tabak. Der Züchter **6**, 25—30 (1934). — 23. SETCHELL, W. A., T. H. GOODSPEED and R. E. CLAUSEN: Inheritance in *Nicotiana tabacum*. I. A report on the results of crossing certain varieties. University of California Pub. in Botany **5**, 457—582 (1922). — 24. WERNSMAN, E. A., and D. F. MATZINGER: A breeding procedure for the utilization of heterosis in tobacco-related species hybrids. Crop Sci. **6**, 298—300 (1966).