

INHERITANCE IN NICOTIANA TABACUM. XXIV.
INTRASPECIFIC DIFFERENCES IN CHRO-
MOSOME STRUCTURE

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THE tobacco of commerce, *Nicotiana tabacum*, is a cultivated tetraploid species which has arisen by hybridization between two diploid species followed by doubling of the chromosome number. When, however, the amphidiploid involving its putative parent species was experimentally produced it was found that certain features were markedly different from the derived species, notably its complete female sterility. It is, therefore, of considerable theoretical and practical interest to determine the nature of the changes which have occurred since the original allopolyploids appeared. One avenue of approach to the problem is a study of the differences in chromosome structure among the numerous varieties. These include a number of more or less improved widely grown commercial types as well as representatives of relatively small local populations, door-yard varieties, etc., the majority of which have been collected in the Andean region of South America. So far as is known there are no wild representatives of the species in the strict sense. Cytological examination reveals the presence of 24 bivalents in all of these varieties, but when they are hybridized, the F_1 hybrids in many instances exhibit associations of four and six chromosomes, indicative of the induction and establishment of reciprocal translocations. MALLAH (1943) first demonstrated and analyzed a few such differences. Since that time many additional varieties have been studied and the identity of most of the translocated chromosomes has been determined.

MATERIALS

In addition to the 15 varieties studied by MALLAH, 40 more recent accessions have been examined in hybrid combination. In general, 26 are of the familiar commercial type with large leaves, rather coarse central stems and terminal inflorescences. One of them, the highly inbred variety *Purpurea*, has been used as a standard of reference throughout the investigation. The other 29 varieties are more weedy in habit and are characterized by the production of relatively small seeds. The latter characteristic has proved of value in selecting varieties to be examined for possible chromosome interchanges. Cytological studies were made on temporary preparations of hybrid anthers stained with aceto-orcein as outlined by CLAUSEN and CAMERON (1944).

VARIETAL CLASSIFICATION ON THE BASIS OF CHROMOSOME HOMOLOGIES

Each of the varieties included in the following account has been crossed with variety *Purpurea* and the resulting hybrids subjected to cytological study.

Whereas translocation complexes are not the only evidences of difference these are the only ones which appear to be amenable to analysis. A decrease in the normal chiasma frequency is indicated in some hybrids and in at least one a heteromorphic bivalent may be recognized in most cells. Anaphase bridges with accompanying fragments suggest the presence of inverted segments in some hybrids.

In table 1 the chromosomal associations of representative varieties in relation to certain selected testers are summarized. Those which exhibit complete bivalent formation in hybrids with *Purpurea* have not been tested further. All the varieties with relatively interchanged chromosome segments have been studied in hybrid combination with *Huadquina* and *Apolo Red*; also *Chinchao* and/or *Cuba White*. Eight varieties are similar to *Huadquina* in their pairing relationships and 11 are included with *Coripata* for the same reason. The latter group requires further explanation. Its members regularly give rise to

TABLE 1
Chromosomal association in varietal hybrids.

	<i>Purpurea</i>	<i>Huad.</i>	<i>Apolo R.</i>	<i>Chinch.</i>	<i>Cuba W.</i>
<i>Coripata</i>	4	4(?)	4+4	4+4
<i>Ceniza</i>	4	4+4	6	6+6	4+4+6
<i>Cerro Alegre</i>	4+4	4	4+6	4+4+4	4+4+4
<i>Pabur</i>	6	4	4+6	4+4+4	4+6
<i>Cuba White</i>	4+6	4+4	4+4+6	4+4+4+4	
<i>Chinchao</i>	4+6	4+4	6+6		
<i>Apolo Red</i>	4	4+4			
<i>Huadquina</i>	4				

a ring of four in hybrids with *Purpurea*. However, in combination with *Huadquina* chromosomes the cytological results are inconsistent. Some of these hybrids have a fairly high frequency of cells with a ring of four, indicating that the same chromosomes are involved but with a different segmental arrangement. In others a difference in structure is revealed only by a failure of complete bivalent formation ($23_{II} + 2_I$). If an association of four were regularly present in *Coripata*-*Huadquina* hybrids, then the combination *Coripata*-*Chinchao* should have two complexes of four and six, but in each case the same result is obtained as that in F_1 *Huadquina*-*Chinchao* (4+4). Further studies on this group are under way.

The varieties which have been investigated are listed below with their places of origin as nearly as it is possible to determine them. The varietal names have no special significance and are used merely for convenience. For more specific identification, accession numbers are included wherever such numbers have been assigned and in most cases these are the UNIVERSITY OF CALIFORNIA BOTANICAL GARDEN numbers. The varieties have been listed in groups corresponding to their above-described cytological relationships.

LARGE SEEDED VARIETIES

<i>Variety</i>	<i>Accession no.</i>	<i>Source</i>	<i>Obtained from</i>
1. <i>Purpurea</i> group			
Ambalema	37-51	Columbia	J. A. Nolla
Apolo Pink	39-246	Bolivia	T. H. Goodspeed
Belge	40-25	Canada	N. A. MacRae
Bergerac catacorolla	40-29	France	H. P. Olmo
Brabazon		Iran	H. P. O.
British Guiana	44-2	Br. Guiana	O. Goodson*
Charpan		Iran	H. P. O.
Colombian M. R.		Colombia	E. E. Clayton
Consolation	40-122	Puerto Rico	J. A. N.
Corrientino 39		Paraguay	Compania Chilena de Tabacos
Corrientino 57		Chile	C. C. de T.
Corrientino 59		Chile	C. C. de T.
Corrientino 60		Chile	C. C. de T.
Criollo palo Amarillo		Argentina	C. C. de T.
Daruma		U. S.	J. Johnson
Girishk		Afghanistan	H. P. O.
Habano Chagres		Paraguay	C. C. de T.
Laguna Sielo	39-32	Argentina	Eyerdam and Beetle*
Maryland mammoth	31g1	U. S.	E. G. Beinhart*
Papago	39-286	U. S.	T. H. G.
Plomo		Chile	C. C. de T.
Purpurea	06-25	U. S.	Missouri Botanic Garden
San Felipe		Chile	C. C. de T.
Serrate	40-23	U. S.	E. E. C.
Station Standup	40-26	Canada	N. A. MacR.
Release Turkish	40-24	U. S.	S. F. Trelease

SMALL SEEDED VARIETIES

<i>Variety</i>	<i>Accession no.</i>	<i>Source</i>	<i>Obtained from</i>
2. Apolo Red	39-245	Bolivia	T. H. G.
3. Ceniza	40-27	Colombia	J. A. N.
4. Cerro Alegre	39-146	Chile	T. H. G.
5. Pabur	39-213	Peru, dept. Piura	T. H. G.
6. Chinchao	36-38	Peru, dept. Huanuco	T. H. G.
7. Cuba White	14-200	Unknown	Mrs. Rose Thomas*
8. Huadquina group			
Amazonas	42-31	Peru, dept. Amazonas	R. D. Metcalf*
Cachicadan	40-42	Peru, dept. Libertad	T. H. G.
Canchaque	39-125	Peru, dept. Piura	T. H. G.
Chachapoyas	42-29	Peru, dept. Amazonas	R. D. M.*
Coquimbo	36-49	Chile	T. H. G.
Cuzco	36-14	Peru, dept. Cuzco	Y. Mexia*
Huadquina	36-170	Peru, dept. Cuzco	T. H. G.

SMALL SEEDED VARIETIES (*continued*)

Variety	Accession no.	Source	Obtained from
Lima	43-29	Peru, dept. Lima	T. H. G.
Sartimbamba	44-4	Peru, dept. Libertad	S. Gastanadui
9. Coripata group			
Acomayo Red	39-184	Peru, dept. Cuzco	T. H. G.
Alameda Red	39-290	U. S.	T. H. G.
Cajamarquilla	45-1	Peru, dept. Libertad	S. G.
Chulque Pink	36-39	Peru, dept. Huanuco	T. H. G.
Chulque Tinged	36-38	Peru, dept. Huanuco	T. H. G.
Coripata	46-48	Bolivia	T. H. G.
Coroica	36-43	Bolivia	T. H. G.
Huanuco	36-58	Peru, dept. Huanuco	T. H. G.
Huaras	45-2	Peru, dept. Huaras	N. van der Walle*
Leimebamba	42-43	Peru, dept. Amazonas	R. D. M.*
Marcapata	39-182	Peru, dept. Cuzco	T. H. G.
Mirador	36-47	Peru, dept. Huanuco	T. H. G.
10. Sandia	42-19	Peru, dept. Puno	R. D. M.*
11. Trujillo	37-4	Peru, dept. Libertad	T. H. G.

*By courtesy of Prof. T. H. GOODSPEED.

MALLAH has already shown that the complexes of Huadquina and Apolo Red are independent and that those of Ceniza and Apolo Red include a common chromosome. In addition, table 1 shows that the same chromosome is present in both the Cerro Alegre and Apolo Red complexes. The other Cerro Alegre translocation is presumably identical or very similar to that of Huadquina. The complex of six in Pabur evidently includes the same translocation that is present in Huadquina. A similar situation was observed in the earlier study with respect to the Huadquina-Cuba White hybrid. Cytological studies of the various hybrids also show that while Chinchao and Cuba White are both characterized by the presence of three translocations involving five chromosomes, some of the chromosomes affected must be different. Two other varieties have been studied incompletely. One, Sandia, has the largest number of translocated chromosomes thus far uncovered, giving three complexes (4+4+6) in hybrids with Purpurea. However, not enough is yet known to place them in their proper relationship to the other varieties.

TYPES OF ASSOCIATION IN THE 4-CHROMOSOME COMPLEXES

Frequencies of different types of association in the 4-chromosome complexes are shown in table 2. In most of these the translocated segments are

TABLE 2
Types of configurations in 4-chromosome complexes in F_1
hybrids involving *Purpurea*.

	Ring of four	Chain of four	Trivalent and single	Two bivalents	One bivalent two singles	Four singles	Total
Huadquina	112	24	9	5	2	1	153
Apolo Red	203	32	1	9	6	1	252
Chinchao	140	69	12	24	5	1	251
Cuba White	135	26	2	1	1	165
Ceniza	9	52	1	91	5	1	159
Cerro Alegre	248/2*	32/2	3/2	24/2	3/2	155

*Two indistinguishable associations of four chromosomes are present in each cell.

apparently of appreciable length, enabling the chromosome arms to form the minimum number of chiasmata required for ring formation. The exception, Ceniza, produces bivalents in the majority of hybrid cells. This variety has the same altered chromosome as Apolo Red and Chinchao, but apparently the breaks have occurred in different regions. The last two classifications are, of course, presumptive as the observed univalents may be the result of chiasma failure in normal bivalents. While this material does not lend itself to a study of chromosome orientation within the rings, it can usually be observed in polar views that the opposed chromosomes lie at a different level of focus. Thus, it seems likely that alternate distributions predominate. This, in combination with the polyploid nature of the species, leads to a very high degree of pollen fertility. Even in heterozygotes with two rings (4 + 6) the percentage of stainable pollen is well within the normal range. *Datura stramonium* (BERGNER *et al.* 1933) and *Triticum monococcum* (THOMPSON and THOMPSON 1937) also may have little pollen abortion in translocation heterozygotes even without the duplication of material afforded by the allopolyploid condition. SNYDER (1951) reports considerable variation in pollen fertility in translocation heterozygotes in *Elymus glaucus*.

IDENTIFICATION OF THE INTERCHANGED CHROMOSOMES

The monosomics of variety *Purpurea* (CLAUSEN and CAMERON 1944) provide a simple though laborious method of identifying the particular chromosomes which take part in the characteristic complexes of the groups delimited above. This was accomplished by crossing selected monosomic plants from the complete series with the following varieties: Apolo Red, Ceniza, Huadquina, Cerro Alegre, Pabur, Chinchao and Cuba White. The resulting populations consist of normal hybrids which show the cytological situation characteristic of the original *Purpurea* hybrids and 47-chromosome plants lacking a normal *Purpurea* chromosome corresponding to the particular monosomic used. If this chromosome is ordinarily a component in a ring of four the constitution of the hybrid will be 22 bivalents plus a chain trivalent. The latter unit will comprise a normal chromosome attached at either end to modified ones. Thus,

in the hybrid *Purpurea*-*Huadquina* the monosomic plants of populations haplo-E and haplo-K give rise to chain trivalents while the $2n - 1$ plants of the other 22 cultures have cells containing $21_{II} + 4 + 1_I$. In the haplo-E culture this trivalent is made up of a normal K chromosome and two translocation products involving E and K chromosomes. *Purpurea* monosomic types haplo-J and haplo-L were isolated by MALLAH (1942) as products of asymmetric distribution from the Cuba White ring of four and the composition of this complex was verified by the present technique. Table 3 shows the make-up of the other complexes which have been investigated. So far the third member of the Chinchao 6-complex has eluded identification and the assignment of the D chromosome to the Pabur association necessarily remains tentative. Further consideration of these features will be reserved for the discussion.

CYTOLOGY OF BACKCROSSES

As we have seen, the chain trivalent present in $2n - 1$ varietal hybrids is presumably made up of a *Purpurea* chromosome associated with two relatively interchanged units. According to expectation, duplication-deficiency gametophytes are nonfunctional and 23-chromosome pollen grains rarely effect fertilization (OLMO 1935). As a result, backcrosses resulting from monosomic *Purpurea* pollinated by corresponding $2n - 1$ hybrids should include only two types. Fertilization of 24-chromosome egg cells should produce plants with meiotic configurations of $22_{II} + 4$ while those developing from 23-chromosome gametophytes should again contain the trivalent.

A study of 32 plants from backcrosses involving the *Purpurea*-*Huadquina* hybrid shows that both interchange chromosomes were present in all fertilizations resulting in disomic plants. However, among the monosomic progenies the results differed depending on which monosomic type was used as pollen parent. Haplo-K *Purpurea*-*Huadquina* individuals gave rise to meiotic configurations of $22_{II} + 3$ in all backcross plants studied. Such backcrosses involved both haplo-E and haplo-K *Purpurea* as ovule parents. When haplo-E *Purpurea*-*Huadquina* plants were used as pollen parents about half the $2n - 1$ individuals studied formed $22_{II} + 3$, and the remainder $23_{II} + 1_I$. This may mean that haplo-E is capable of relatively high transmission through the pollen. However, in no instance where haplo-K *Purpurea* was pollinated by haplo-E *Purpurea*-*Huadquina* were the derived monosomic plants classed as

TABLE 3
Identification of the interchanged chromosomes.

	"tomentosa"				"sylvestris"		
Apolo Red					Q		S
Ceniza							S Z
Huadquina	E		K				
Cerro Alegre	E		K		P Q		
Pabur	(D) E		K				
Chinchao	E		K	? N			S
Cuba White	E	J	K	L		R	

haplo-E on the basis of morphological criteria. The size of the univalent chromosome in cells where the trivalent was replaced by a bivalent and univalent also indicated that the 23-chromosome gamete was contributed by the female parent.

Backcross populations involving hybrids characterized by two associations ($19_{II} + 4 + 6$) were also produced. Depending on the particular monosomic used as ovule parent it would be expected that the ring of four or six would be replaced by a trivalent or chain of five, respectively, in appropriate crosses. *A priori*, one would expect the remaining association to be replaced by bivalents in about half the individuals since gametes with "standard" chromosomes as well as those with "interchanged" ones would be equally viable. This has only been observed in two of 23 such backcross individuals studied. Apparently selective influences favor the retention of the heterozygous condition. This feature should be followed up using larger numbers and reciprocal combinations.

DISCUSSION

Many of the genetic features of *N. tabacum* are known to be governed by duplicate genes (CLAUSEN and CAMERON 1944, 1950) and in each case one has been located in the "sylvestris" and one in the "tomentosa" genome. Also, cytological studies of the diploid hybrid involving these species have revealed a small amount of loose chromosome pairing ($0-7_{II}$). Thus, it might be expected that reciprocal translocations would arise by some process analogous to crossing over in small segments of otherwise nonhomologous chromosomes in the two sets. The present study, however, reveals that in each ring of four investigated thus far, both altered chromosomes are members of the same genome, chromosomes A-L representing the tomentosa contribution. Four of the rings are composed of sylvestris chromosomes (N-S, P-Q, Q-S, S-Z) and two include chromosomes from tomentosa (E-K, J-L). The 6-complex of Cuba White is unique in this respect, having chromosomes from both genomes (E-K-R).

Since the E-K translocation appears in six of 11 associations that have been investigated, it is conceivable that in reality, these two chromosomes have become modified in the line which we have used as standard and in those related most closely to it. Suggestive evidence is provided by the fact that these two chromosomes are at the extremes of size variability within the variety *Purpurea*. Also it is known that a single ring of four is formed in hybrids between this variety and the experimentally produced amphidiploid, $4n$ -sylvestris-tomentosa. It will be of interest to examine the hybrids between this amphidiploid and other varieties such as *Huadquina* or some member of the *Coripata* group.

Assuming that the *Purpurea* group is characterized by the presence of modified E and K chromosomes, the various translocation complexes under study could have arisen by means of a single interchange for each complex. *Huadquina* or *Coripata* and their counterparts would then become the "stand-

ard" line, with Apolo Red and Ceniza each differing from it by two independent interchanges one of which involves a common chromosome (S). A single translocation would account for the ring of four by which Cerro Alegre differs from Huadquina. On this hypothesis the 6-complex of Pabur would be produced by a translocation involving an E or K chromosome and a chromosome as yet not positively identified. The more complex varieties, Chinchao and Cuba White could have arisen as double interchange products, the two rings of four being of independent origin in each case. Preliminary results indicate that Trujillo is probably closely allied to Cerro Alegre. The variety Sandia shows a minimum of chromosomal association in hybrids involving Huadquina but analyses of tester hybrids are admittedly inadequate to place this complex variety in its proper relationship.

Although the varieties Chinchao and Pabur have been subjected to analysis using all 24 of the monosomic types, one member of a chromosomal association of each remains unidentified. There is, therefore, a strong possibility that the monosomic cultures are not completely of the primary type. At first, the D chromosome was regarded as being a member of the 6-complex of Pabur but subsequent studies have indicated that in ring-forming hybrids generally, haplo-D cells are frequently characterized by a breakdown of the ring of chromosomes. In haplo-D *Purpurea* the P.M.C. contain a high frequency of configurations of $22_{II} + 3$. The extra chromosome in each generation is introduced from the normal inbred line yet the condition persists. If it should become possible to obtain the primary type corresponding to this presumably modified monosomic the situation outlined above may be resolved.

SUMMARY

Differences in chromosome structure among 55 varieties of *N. tabacum* have been analyzed in an attempt to obtain further information as to the genetic composition of the species. Hybrids between *Purpurea*, a highly inbred line, and the other 54 varieties reveal that the chromosomes of 25 large-seeded, more or less commercial types are structurally similar to those of *Purpurea*. Homozygous, relatively interchanged chromosomes are present in 29 varieties which are characterized by relatively small seeds and weedier growth habit.

Cytological studies of hybrids between four selected tester varieties and the above 29 give evidence of 11 different classes with regard to the configurations obtained. These involve ten different complexes of four and six chromosomes.

Monosomic analysis of the complexes has made possible the identification of all but two of the interchanged chromosomes. In nearly all cases the components of a particular association are members of the same genome.

Results of cytological studies of backcrosses indicate that transmission of certain monosomics through the pollen may be higher than expected on the basis of previous work.

Consideration is given to the problem of the structural type from which the other varieties have been derived.

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