A CYTOGENETIC STUDY OF HERITABLE TUMORS IN NICOTIANA SPECIES HYBRIDS¹

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GENETIC tumors in *Nicotiana* species hybrids have been reported by several investigators. These tumors are formed in certain specific hybrid combinations on stems, roots, and occasionally on leaves of all or part of the F_1 progeny. Since the discovery by KOSTOFF (1930) of nine interspecific tumorous hybrids, almost 20 additional hybrid combinations have been reported in which the entire progeny is tumorous. In 23 additional species combinations, hereditary tumors are produced irregularly in some of the progeny or restricted to only certain parts of the plants (KEHR and SMITH 1954; SMITH 1958). Furthermore, it was noted that *Nicotiana langsdorffii*, *N. glauca*, *N. rustica* and *N. alata* enter into more tumorous hybrids than other *Nicotiana* species. NÄF (1958) went a step further and tentatively and arbitrarily divided certain species of *Nicotiana* into two groups, namely plus group and minus group; tumors are formed when the species involved are from opposite groups. Thus, according to him, the plus group consists of *N. forgetiana*, *N. langsdorffii*, *N. sanderae*, *N. alata*, *N. longiflora*, *N. noctiflora*, *N. bonariensis*, and *N. plumbaginifolia*; the minus group species are *N. tabacum*, *N. bigelovii*, *N. suaveolens*, *N. miersii*, *N. paniculata*, *N. debneyi*, *N. glauca*, and *N. rustica*. It is of interest to note that seven of the eight plus group species (all but *N. noctiflora*) belong to the section *Alatae*.

Morphologically, several types of tumors can be recognized. BURK and Tso (1960), working on the cytology of tumors, have described, on the basis of their appearance, two specific types of tumors, that is, "Pseudo" and "Cauliflower," in F_1 *N. glauca* $\times$ *N. langsdorffii* ($2n = 21$). The "Pseudo" form had a characteristic chromosome number of 19 and produced leaf-like structures, while "Cauliflower" types produced only small shoot-like protuberances a few millimeters in length and characteristically had a chromosome number of 18. According to these authors, chromosome loss may be the cause rather than the result of genetic plant tumors.

Cytogenetic studies of KEHR and SMITH (1954) have shown that tumors are produced on any diploid, triploid, or tetraploid combination of *N. glauca* ($n = 12$) and *N. langsdorffii* ($n = 9$) possessing at least one complete genome of each of the parent species. In an effort to establish an association between tumor formation and particular chromosomes, KEHR and SMITH recurrently backcrossed

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amphidiploid *N. glauca-langsdoeffii* to *N. langsdoeffii*, accompanied by selection for extra alien chromosomes of *N. glauca*. However, progeny with less than a full genome of *N. glauca* in combination with two of *N. langsdoeffii* produced no tumors. KEHR and SMITH did not examine the cytology at meiosis or mitosis in the backcross progeny. Recently, IZARD (1957) has been able to obtain two tumorless lines derived from X-rayed seed of amphidiploid *N. glauca-langsdoeffii* which had a complete complement of 42 chromosomes and 80 to 100 percent good pollen. This study supports the idea of genetic control of tumor production.

The present investigation was undertaken to find out whether tumor production depends upon genomic (complete chromosome set) control in other than *N. glauca* $\times$ *N. langsdoeffii* hybrids and hybrid derivatives.

MATERIALS AND METHODS

A number of diploid as well as artificially produced amphidiploids of *Nicotiana* species have been employed in the current study. *N. tabacum* ($n = 24$) is placed in subgenus *Tabacum*, section *Genuinae* (GOODSPEED 1954). *N. langsdoeffii* ($n = 9$), *N. alata* ($n = 9$), *N. longiflora* ($n = 10$), *N. forgetiana* ($n = 9$), *N. bonariensis* ($n = 9$), and *N. plumbaginifolia* ($n = 10$) all belong to the subgenus *Petunioides*, section *Alatae*. These six 9-paired and 10-paired species are relatively closely related and exhibit a high degree of chromosome homology when crossed with each other. *N. glauca* ($n = 12$) belongs to subgenus *Rustica*, section *Paniculatae*. The $4n$ *N. tabacum* ($n = 48$) is an artificially produced autotetraploid. It has coarser and smaller flowers and is also reduced in height in comparison with the diploid. The three amphidiploids, $4n$ *N. undulata-tabacum* ($n = 36$), $4n$ *N. debneyi-tabacum* ($n = 48$), and $4n$ *N. longiflora-tabacum* ($n = 34$), were also artificially produced.

For cytological analysis, young buds, after removing calyx and corolla, were fixed in acetic-alcohol (1:3) for 24 hours and subsequently stored in 70 percent alcohol. Anthers were smeared in acetocarmine and the cover glass, after being gently pressed to remove the excess of stain, was sealed with paraffin. The slides were made permanent the following day by the technique of BHADURI and GHOSH (1954). Chromosome counts were made at first metaphase of pollen mother cells. Pollen sterility of hybrids and their derivatives were determined by staining pollen grains with acetocarmine and scoring the unstained, shriveled and empty grains.

The interspecific backcross method was used for eliminating all but a few or one of the chromosomes of one species involved in the hybrid combination. Essentially this method consists either in crossing the amphidiploid with a diploid species and then repeatedly backcrossing the F_1 to the amphidiploid until one of the alien chromosomes is isolated in the otherwise amphidiploid nucleus, or repeatedly backcrossing the tumorous amphidiploid with one of the parental diploid species. Tumor extracts were applied on normal healthy plants to test for presence of a tumor inducing agent. In addition grafts of nontumorous on tumorous stock and *vice versa*, were made. Reciprocal grafts between *Nicotiana glauca* and *N. langsdoeffii* were also observed.

Microphotographs were taken using a Bausch and Lomb K camera at an initial enlargement of 1200 $\times$.

RESULTS

A number of interspecific hybrids of the genus *Nicotiana* were made in the present study with the view of studying cytogenetics of tumor formation in a variety of materials. Owing to sterility barriers in backcrosses at the diploid level, only two of six combinations, that is, 4n *N. debneyi-tabacum* $\times$ *N. longiflora* and 4n *N. longiflora-tabacum* $\times$ *N. tabacum* were favorable and these form the basis of this report.

TABLE 1

Hybrids in which germination failed or seedlings died

Species hybrid	No. of seeds sown	No. of seeds germinated
<i>N. glauca</i> $\times$ <i>N. forgetiana</i>	ca. 1000	0
*4n <i>N. tabacum</i> $\times$ <i>N. langsdorffii</i>	50	41
4n <i>N. tabacum</i> $\times$ <i>N. alata</i>	50	11
4n <i>N. tabacum</i> $\times$ <i>N. forgetiana</i>	ca. 1000	0
4n <i>N. debneyi-tabacum</i> $\times$ <i>N. alata</i>	50	7
4n <i>N. debneyi-tabacum</i> $\times$ <i>N. forgetiana</i>	50	0
†4n <i>N. debneyi-tabacum</i> $\times$ <i>N. langsdorffii</i>	ca. 1000	30
4n <i>N. debneyi-tabacum</i> $\times$ <i>N. bonariensis</i>	50	0
4n <i>N. undulata-tabacum</i> $\times$ <i>N. longiflora</i>	50	0
4n <i>N. undulata-tabacum</i> $\times$ <i>N. bonariensis</i>	ca. 1000	4
†4n <i>N. undulata-tabacum</i> $\times$ <i>N. alata</i>	ca. 1000	8
4n <i>N. undulata-tabacum</i> $\times$ <i>N. langsdorffii</i>	ca. 1000	25

* Seedling roots were yellow.

† See text.

Table 1 summarizes the results of crosses (where one of the species belongs to section *Alatae*) in which tumors may be expected on the basis of previous results. In all these instances either the seeds did not germinate or the seedlings were inviable. Nevertheless, one hybrid combination (4n *N. debneyi-tabacum* $\times$ *N. langsdorffii*) was of special interest. In this instance, also seedling lethality ensued, but when 4n *N. debneyi-tabacum* having an alien *longiflora* chromosome (that induced tumor formation) was crossed to *N. langsdorffii* many seeds were obtained. The germination of such seeds was excellent but the great majority died and had seedling tumors (Figure 1). However, a few mature plants were obtained. Presumably, a buffering effect of the *longiflora* chromosome was responsible for such a difference. This phenomenon is being studied further and will be discussed elsewhere.

*F*₁ 4n *N. debneyi-tabacum* $\times$ *N. longiflora*: A large number of plants of this cross were raised in the greenhouse. Presence of seedling tumors caused considerable mortality at this stage. As a result, only six plants reached maturity. Tumors, which were mostly restricted to nodes of stems, appeared on these plants before and after flowering. No tumors were observed on leaves or flowers. The

FIGURE 1.—Seedling tumors (arrows) in the hybrid ($4n$ *N. debneyi-tabacum* with an alien *longiflora* chromosome) $\times$ *N. langsdorffii*.

FIGURE 2.—Tumors (in various stages of development) on plants of $4n$ *N. debneyi-tabacum* $\times$ *N. longiflora*. Scale is in centimeters.

flowers were complete and hermaphroditic. There was, however, almost 98 percent pollen abortion in all the plants. The reciprocal cross was not obtained. Cytologically, meiosis was very disturbed. There were a few bivalents, probably from pairing between *tabacum* and *debneyi* chromosomes because pairing between *longiflora* chromosomes and those of either *tabacum* or *debneyi* is a rare event.

First backcross generation: These plants were derived by crossing $3n$ *N. debneyi-tabacum-longiflora* with $4n$ *N. debneyi-tabacum*. Presumably as a result of highly irregular meiosis in the $3n$ hybrid, very few flowers yielded fruits when backcrossed. The seed-set was very poor and only 17 plants could be raised to maturity; of these, 12 had tumors. One of the nontumorous plants looked like normal $4n$ *debneyi-tabacum* and had perfectly stainable pollen; a study of meiosis revealed a constitution of $46_{II} + 2_I$.

Pollen abortion was high in the remaining 16 plants of the population; the pollen stainability ranged from 0–20 percent. In addition to tumors, which may be looked upon as manifestations of instability (MOAV and CAMERON 1960), flower variegation was quite pronounced. Analysis of meiotic configurations in these plants was more readily accomplished than in the F_1 progeny. Meiotic configurations of a few plants revealed that there are six to eight chromosomes of *N. longiflora* that remained in plants after the first backcross. PMC of one plant of the first backcross generation is shown in Figure 3.

Second backcross generation: Two plants from the first backcross (b.c.) generation with constitutions of $(47_{II} + 8_I)$ and $(48_{II} + 6_I)$ were crossed to $4n$ *debneyi-tabacum*. A total of 108 plants were raised in the greenhouse in two

FIGURE 3.—PMC of a plant from the first b.c. generation with $48_{II} + 6_I$.

FIGURE 4.—MI configuration in a third b.c. generation plant with $46_{II} + 2_I + 1$ fg. (arrow) (fg = chromosomal fragment).

FIGURE 5.—Diakinesis showing association between the fragment and the alien *longiflora* chromosome (arrow).

FIGURE 6.—MI configuration in a first b.c. generation plant $4n$ *N. longiflora-tabacum* $\times$ *N. tabacum* showing $24_{II} + 2_I + 2$ fgs. (arrows).

seasons, that is, in the winter of 1959 and the summer of 1960. About half of the plants in both seasons showed tumors.

Tumors occurred both at nodes and on the internodes of stems (Fig. 2). Nodal tumors were variable in size and shape. They were normally green in color but often showed pink areas, probably due to the presence of anthocyanin. The nodal tumors were usually completely undifferentiated or on occasions produced shoots with rudimentary flowers. The internodal tumors, on the other hand, were very small papilla-like projections and were frequently not more than a few millimeters in diameter. These were green, mostly undifferentiated and usually maintained a constant size. Sometimes they covered the whole stem.

In order to determine how many *longiflora* chromosomes must be present along with those of *debneyi-tabacum* and still give tumors, the cytology of tumorous as well as nontumorous plants was examined. It was found that in the tumorous plants of the second b.c. generation the *longiflora* contribution was reduced either to a chromosome fragment or as many as three univalents. *Longiflora* chromosomes may be identified under the microscope by their larger size as compared to those of *tabacum* or *debneyi*. It was found that in the nontumorous plants of the second b.c. generation one or two *longiflora* chromosomes were also present. From this it was concluded that not all the *longiflora* chromosomes were capable of inducing tumor formation. Perhaps it is reasonable to assume that there is a specific *longiflora* chromosome carrying a tumor inducing region. This hypothesis was borne out by further experimental data.

Since few genetic loci have been identified in *longiflora* chromosomes, an attempt was made to find other factors located on the chromosome carrying the tumorous trait. Two such characters, each presumably controlled by a single gene, were observed in the tumorous plants. These were presence of anthocyanin in the corolla and abortive pollen. Cytologically, the tumorous plants with flowers containing anthocyanin showed two *longiflora* univalents in hybrid cells. Plants with a single *longiflora* chromosome or a *longiflora* fragment exhibited no anthocyanin in their flowers. An extensive study was made of pollen abortion in the initial hybrid and subsequent backcross generations, because this approach seemed to hold promise of yielding results of interest.

One population was derived by crossing a first b.c. generation plant ($47_{II} + 8_I$) to $4n$ *debneyi-tabacum*. Only four plants were examined cytologically. One had no tumors and no *longiflora* chromosomes, while of the remaining three plants, which were all tumorous, one possessed two *longiflora* chromosomes, the second a *longiflora* fragment and the third three *longiflora* chromosomes.

The second population was obtained by crossing a first b.c. generation plant ($48_{II} + 6_I$) with $4n$ *debneyi-tabacum*. Of the 32 plants grown 24 were studied cytologically and the results are summarized in Table 2. Thirteen tumorous plants showed in their PMC a single fragment, one *longiflora* chromosome, two *longiflora* chromosomes or two *longiflora* chromosomes plus a fragment. Unfortunately, pollen fertility data were not recorded for all the plants. Only three tumorous plants were scored for pollen fertility. One showed two *longiflora* chromosomes at MI and had 48.3 percent stainable pollen. There was only one

TABLE 2

MI configurations in tumorous and nontumorous second backcross generation plants

Plant no.	Tumors	<i>longiflora</i> chromosomes	Anthocyanin
1	+	2	+
2	..	2	+
3	..	2 + 1 fg*	..
4	..	2	+
5	+	2 + 1 fg	+
6	..	..	..
7	..	2	+
8	+	1	..
9	+	1 fg	..
10	..	..	..
11	+	1 + 1 fg	+
12	+	2 + 1 fg	..
13	+	2	..
14	+	2	+
15	..	1	+
18	+	1 + 1 fg	..
19	..	2 + 1 fg	..
20	+	2 fgs	+
22	+	2	+
23	+	2 + 1 fg	+
28	+	2	+

* fg = chromosomal fragment.

longiflora chromosome in the second plant and it had completely aborted pollen. The third had 90 percent stainable pollen and contained one *longiflora* fragment. These last two plants did not have anthocyanin pigment in the corolla, indicating that the anthocyanin controlling gene (or genes) is located elsewhere in the *longiflora* genome.

The third backcross generation. Several plants from the second b.c. generation were crossed with 4n *debneyi-tabacum*. Six plants were selfed or sib-crossed. A total of 308 plants were raised to maturity in the third b.c. generation.

The hypothesis that there may be an alien *longiflora* chromosome which, in otherwise *debneyi-tabacum* nuclei, induces tumor production was substantiated by evidence from the third b.c. generation. Furthermore, it was observed that the majority of tumorous plants had abortive pollen. Consequently, it was assumed that tumor inducing and pollen abortive regions are located on the same *longiflora* chromosome.

Eight populations were derived from the second b.c. tumorous maternal parents with *longiflora* contributions ranging from two *longiflora* chromosomes and a fragment to one *longiflora* fragment. Out of a total of 159 plants 55 plants were tumorous (34.6 percent transmission of the tumorous trait).

Four classes of plants may be recognized. Class I contains tumorous and pollen sterile plants. Class II consists of tumorous and pollen fertile plants. Class III is

composed of nontumorous and pollen sterile plants and class IV is composed of normal plants.

Several plants were examined cytologically and the results in the four classes were: class I plants had an alien *longiflora* chromosome in addition to the 4n *debneyi-tabacum* complement. Class II contained a centric fragment of the alien *longiflora* chromosome presumably carrying the tumor causing segment but lacking the fragment associated with pollen sterility (Fig. 4). Class III plants also contained a centric fragment presumably with the tumor inducing region eliminated. A few plants in class IV have a centric fragment of the *longiflora* chromosome, supposedly with neither of the two regions under study. However, most of the plants in this class had no *longiflora* chromosomal material. There were only two exceptions to the general hypothesis. One tumorous plant contained a *longiflora* univalent in PMC in addition to the complete set of 4n *debneyi-tabacum* chromosomes but showed 75 percent stainable pollen. This condition could have arisen by somatic elimination of the *longiflora* chromosome from the branch bearing such flowers. The second plant was nontumorous and highly pollen-sterile (90 percent sterility), and did not show a fragment of the *longiflora* chromosome. Such a condition may perhaps have either arisen due to incorporation of the fragment presumably carrying the pollen-sterility segment into the *debneyi-tabacum* genome as a result of interchange or perhaps the pollen abortion segment was too small to demonstrate.

One of the third b.c. families had a relatively high transmission of the *longiflora* chromosome. In this population, derived by backcrossing a second b.c. maternal parent (having two *longiflora* chromosomes and a fragment) to 4n *debneyi-tabacum*, there were 13 tumorous and three nontumorous male-sterile plants out of 20. It would appear, therefore, that 16 of 20 plants were carrying a *longiflora* chromosome or its fragment (Table 3, culture 9). This high transmission of the alien chromosome is more than double the normal transmission of other b.c. generations. Such a high rate could have resulted if the seed parent of this cross carried two homologous *longiflora* chromosomes with the tumor inducing and pollen-sterility regions. The seed parent contained two *longiflora* chromo-

TABLE 3

Four classes of third backcross generation plants based on tumor production and pollen-sterility

Cultures	Total no. of plants	I Tumorous and pollen sterile	II Tumorous and pollen fertile	III Nontumorous and pollen sterile	IV Normal
1	24	4	3	1	16
2	20	3	0	1	16
3	20	4	4	1	11
4	15	0	3	1	11
5	20	6	4	1	9
6	20	3	5	0	12
7	20	0	9	0	11
8	20	0	7	0	13
9	20	7	6	3	4

somes and a fragment and two of these three elements may have been homologous if nondisjunction occurred in the first b.c. generation. If this explanation is correct, one would expect to find twice as many plants with a *longiflora* chromosome or fragment as compared to the other families. This was confirmed by cytological analysis. Meiosis was studied in a total of ten tumorous and nontumorous plants and the results are listed in Table 4.

Selfed derivatives of the second backcross generation: Selfed progenies of three second b.c. generation plants yielded essentially the same results as the third b.c. generation, that is, tumorous and pollen abortive plants, tumorous and pollen fertile plants, nontumorous and pollen sterile plants and normal plants. The cytology in seven of these plants was in agreement with the results from the third b.c. generation plants, that is, class I carried an alien chromosome, classes II and III carried the centric fragment of the alien chromosome and class IV contained practically no alien material.

Evidence that a particular longiflora chromosome is associated with tumor production: In the third b.c. generation, in general, the contribution of *longiflora* chromosomes was reduced to one or a fragment of a chromosome. The next problem was to find out whether a specific *longiflora* chromosome was involved. For this purpose, sib-crosses between three plants of the second b.c. generation were made.

Since the great majority of the tumorous plants had aborted pollen, the crosses had to be planned in such a way that one of the parental tumorous plants was male fertile. Such tumorous male-fertile plants do occur and these contain the *longiflora* fragment with the tumor producing region but not the sterility producing region. Reciprocal crosses were made between two second b.c. plants, one with two *longiflora* chromosomes and the other the *longiflora* fragment. Another population was derived by crossing a second b.c. plant which contained the *longiflora* chromosome with another (having a fragment). A few plants were examined for pairing between the *longiflora* chromosome and the fragment at diakinesis. Since there is very little transmission of the extra chromosome through

TABLE 4

MI configurations of tumorous and nontumorous plants of an exceptional third backcross population

Plant no.	Tumor	<i>longiflora</i> chromosome material	Percent stainable pollen
1	+	1 fg	70
2	..	none	75
4	..	1 fg	10
7	+	1*	14
8	..	1 fg	10
14	..	none	50
17	..	1 fg	50
19	..	1 fg	66
20	+	1*	10

* Single chromosome or a very large fragment.

the pollen, it was more difficult to choose plants with both *longiflora* elements, that is, the *longiflora* chromosome and the fragment. However, one plant, which was tumorous and had 97 percent abortive pollen, did show an association between the alien chromosome and the fragment (Figure 5).

Association of tumor production and pollen sterility: Six third b.c. populations (see Table 3, cultures 1–6) were classified, on the basis of stainable pollen, into eight classes with intervals of 12.5 percent (Figure 7). Two things are clearly evident from this histogram. First, the pollen fertility data present a bimodal curve with a low point at about 30 percent. Therefore, it was decided to group all plants below 30 percent in a low fertility group and all above this level in a high group. Second, it can be seen that very high percentages of tumors are present in the low fertility group.

A chi-square test showed that the first two classes with high tumor incidence are homogeneous with respect to tumor formation and the remaining six classes with low tumor formation are also homogeneous with respect to each other. In addition, a 2×2 contingency test of the four classes mentioned in Table 3 (cultures 1–6) gave a chi-square of 25.8 which is very highly significant ($P \ll .001$), and shows a definite relationship between tumor production and pollen sterility. This further supports the hypothesis that both factors may be located on the same chromosome.

FIGURE 7.—Histogram showing relationship between presence of tumors and pollen sterility.

Transmission of the alien chromosome: In the third b.c. families it was observed that in a great majority of plants the number of *longiflora* chromosomes was reduced to only one or to a fragment. This would indicate that an alien *longiflora* chromosome influences the tumorous trait. Also, all plants carrying the whole alien chromosome were tumorous and male-sterile. Male-fertile and tumorous plants were quite frequent in several families and these contained only a fragment of the alien chromosome. Consequently, one would expect to obtain male-sterile, nontumorous plants having the remainder of the same chromosome. Several male-sterile, nontumorous plants were cytologically analyzed and all but one possessed a fragment. Since fragments of the alien chromosome are frequently seen at meiosis, it is reasonable to assume that transmission rates based on the tumorous trait alone would give a low estimate. Data including both pollen sterility and tumor production would give a more accurate value of the transmission of the alien chromosomal material. Depending on location of breaks, four classes are theoretically possible: (1) tumorous, male-sterile, (2) tumorous, male-fertile, (3) nontumorous, male-sterile and, (4) nontumorous male-fertile. A classification based on tumor production and pollen sterility is given in Table 3. Individual plants of two populations of the second b.c. generation were backcrossed to 4n *debneyi-tabacum*, and the plants listed in Table 3 represent the third b.c. generation. In order to make a chi-square test of transmission of the alien chromosome, the first three classes, that is, tumorous-sterile, tumorous-fertile and nontumorous-sterile were lumped, since the number of plants in each class of the populations listed in Table 3 (culture 1-6) was less than five. The transmission rate of classes I, II and III of the 119 plants of different families in Table 3 (cultures 1-6) was 37 percent and the families were found to be homogeneous for this transmission rate ($P = 0.3-0.2$).

Probably the strongest evidence for the hypothesis that a region of the alien chromosome is responsible for tumor production comes from two populations of the third b.c. generation (Table 3, cultures 7, 8). These populations were derived from plants which were tumorous and pollen fertile and, consequently, these plants only transmitted the centric fragment causing tumor formation. Transmission of this fragment was 40 percent which approximates the 37 percent transmission of the whole *longiflora* chromosome.

Selfing and sib-crosses gave a transmission of 52.3 percent for classes I, II, and III (57 of 109) as compared to 37 percent in the backcrosses (44 of 119). When a chi-square test for independence is applied to the first three classes in selfing and backcrossing, the differences are significant ($P = 0.02$) and suggest the occurrence of some male transmission. In the absence of the critical cross, using tumorous, pollen-fertile plants as male parents with 4n *debneyi-tabacum*, an accurate estimate of male transmission of the alien material cannot be given at this time. Data are being gathered for this purpose.

The transmission rate of about 40 percent in our material is comparable to the approximately 50 percent ovular transmission of necrotic mosaic resistance reported by GERSTEL (1945) in certain sesquidiploids of *N. tabacum-glutinosa*. However, in the same paper he reports that transmission of a single *glutinosa*

chromosome was about 16 percent through ovules and 14 percent through the pollen. CHU (1954), working with a dominant marker from *glutinosa* controlling chlorophyll production in the above cross, obtained a transmission of 20 percent through the ovules.

F_1 $4n$ *N. longiflora-tabacum* $\times$ $2n$ *N. tabacum*: One approximately amphidiploid *longiflora-tabacum* plant had tumors and perfectly good pollen. Cytological analysis revealed that its constitution was $31_{II} + 3_I$. This plant was backcrossed to diploid *tabacum* and the F_1 consisted of 20 plants, out of which eight were tumorous. The pollen was uniformly abortive in all the plants. Meiosis was studied in four plants. The three tumorous ones showed at MI $24_{II} + 8_I$, $24_{II} + 8_I$ and $24_{II} + 7_I$. The nontumorous plant's constitution was $24_{II} + 7_I$.

First backcross generation: Two plants of the F_1 were backcrossed to diploid *tabacum*. Only one surviving plant in one culture was nontumorous and had 85 percent apparently good pollen.

Thirty-two plants were grown in the second culture, of which 12 plants were tumorous (37.5 percent transmission of tumors). The cytology of ten plants was examined and is listed in Table 5. PMC of one plant (p29) is shown in Figure 6. It can be seen that tumor production and pollen sterility are not associated as in $4n$ *debneyi-tabacum* $\times$ *longiflora*.

Grafting experiments: In order to test for presence of a transmissible agent two conventional methods were employed. Tumorous F_1 (*N. glauca* $\times$ *N. langsdorffii*) was reciprocally grafted using normal *N. glauca*. Likewise, two tumorous plants (third b.c. population of $4n$ *debneyi-tabacum* plus a *longiflora* fragment) were reciprocally grafted with $4n$ *debneyi-tabacum*. In both cases there was no apparent movement of any growth promoting substance across the graft union. Tumor extract when applied to the cut surfaces of stems of normal *N. glauca* or *N. langsdorffii* did not induce any tumors.

That tumors are autonomous was confirmed by grafting a normal looking scion from F_1 —*N. glauca* $\times$ *N. langsdorffii*—on to *N. glauca*. The scion developed

TABLE 5

MI configurations of tumorous and nontumorous plants of the first backcross generation of 4n N. longiflora-tabacum $\times$ N. tabacum

Plant no.	Tumor	longiflora chromosomes	Percent stainable pollen
1	+	2	95
16	+	1	94.5
19	+	1	95
22	+	2	60
24	+	4	8
29	+	2 + 2 fgs	15
30	+	2	50
32	+	4	85
7	..	2	95
15	..	1	80

characteristic bead-like tumors all over the stem at a later date without affecting the stock.

Reciprocal grafts were made between *N. glauca* and *N. langsdorffii*. No tumors were seen on the stocks or the scions. However, small tumor-like projections were noticeable at the place of union of grafting when leaves and buds were removed from the stocks and scions of such grafts. This may mean that both species have some growth substances which normally do not move across the union but interact to form tumor-like projections at the point of union.

DISCUSSION

Cytogenetics and tumor production: Since it is widely recognized that tumors may be produced at will by appropriate interspecific hybridizations in *Nicotiana*, the next step was to determine the nature of the hereditary control. The first report in this regard was published in 1954 by KEHR and SMITH. They made a considerable number of diploid and polyploid combinations involving the two species, *N. glauca* and *N. langsdorffii*. Genome combinations which they obtained ranged from an extreme of two *langsdorffii* genomes to one of *glauca* to the other extreme of one genome of *langsdorffii* to three genomes of *glauca*. Tumors were conspicuous on all parts in all combinations. Based on these data the authors concluded that the tumor forming nature of the hybrid, *N. glauca* $\times$ *N. langsdorffii* remains unchanged regardless of the ratio of *glauca* and *langsdorffii* genomes, as long as at least one complete genome of each species is present in the hybrid. No exceptions were found, even in the multiple genome combinations involving up to four species.

In an effort to establish an association of tumor formation with particular chromosomes, KEHR and SMITH (1954) backcrossed amphidiploid *N. glauca-langsdorffii* ($n = 21$) to *N. langsdorffii* ($n = 9$) and backcrossing was continued, accompanied by selection for extra alien chromosomes of *N. glauca*. No progeny with less than a full genome of *N. glauca* combined with *N. langsdorffii* produced tumors.

Recently SMITH and STEVENSON (1961) have extended the genetic analysis to substantiate evidence for genetic control. Segregation and linkage with a polygenic marker system were demonstrated. Their results favored an interpretation that a number of genes throughout the complement of *N. langsdorffii* govern tumor formation and are required for its expression. This is in line with the earlier finding that with only one or a few *glauca* chromosomes in *langsdorffii-glauca* hybrid derivatives tumors do not form.

Cytogenetic analysis along the same lines as mentioned above has been presented in this paper, one of the more promising interspecific crosses being $4n$ *N. debneyi-tabacum* ($n = 48$) $\times$ *N. longiflora* ($n = 10$). The F_1 progeny was tumorous. By repeated backcrossing to $4n$ *debneyi-tabacum* for three generations accompanied by selection for tumors, it was found by cytological analysis, that there is a *longiflora* chromosome which when present in otherwise *debneyi-tabacum* induces tumor production. Even a fragment, derived from the alien

chromosome, induces tumor formation. A segment of the same chromosome was found to be associated with pollen abortion.

Similar cytogenetic analyses of a b.c. progeny of $4n$ *N. longiflora-tabacum* ($n = 34$) $\times$ *N. tabacum* ($n = 24$) for two generations revealed that in this case too a single *longiflora* chromosome on a *tabacum* background can induce tumors. Thus it is clear that the whole genome is not required for tumor production in all instances. It is of interest to note that the radiation induced mutant of IZARD shows some evidence of simple genetic control (SMITH and STEVENSON 1961).

Chromosome breaks and pollen sterility: Every organism presumably has its own rate of spontaneous chromosomal change, a rate governed by both intrinsic and extrinsic factors (SWANSON 1957). GILES (1940, 1941) studied extensively the rate of spontaneous chromosomal aberrations in interspecific hybrids between *Tradescantia paludosa* and *T. canaliculata*. He found that frequency of aberrations increased threefold in F_2 as compared to the parental species.

In the $4n$ *N. debneyi-tabacum* $\times$ *N. longiflora* hybrid and hybrid derivatives a fairly high frequency of fragments of the *longiflora* chromosomes resulting from chromosomal breaks occurred in the second b.c. generation (Table 2). Since tumor and pollen-sterility controlling regions are located on the same *longiflora* chromosome, it was fairly easy to follow the consequences of fragmentation of this chromosome through subsequent generations. As mentioned earlier, the cytogenetic evidence confirms the hypothesis that spontaneous breakage of the *longiflora* chromosome occurs on a *debneyi-tabacum* background. There are four possible classes on the basis of morphology of plants resulting from breaks at different points along this chromosome. Class I involves no breaks and includes plants having the whole chromosome, that is, tumor production and pollen sterility. Class II consists of tumorous plants only, in which the sterility region is presumably lost. Class III consists of nontumorous but pollen-sterile plants and contains the centric fragment in which the tumor inducing region is supposedly lost. Class IV is composed of normal plants. A few plants in class IV have a centric fragment of the *longiflora* chromosome presumably with neither of the regions under study. However, most of the plants in this class did not carry any portion of the alien chromosome.

The behavior of probably the same *longiflora* chromosome when combined with two different genomes, namely, $4n$ *debneyi-tabacum* and $2n$ *tabacum* is of interest. This chromosome in *debneyi-tabacum* causes pollen abortion, whereas in diploid *tabacum* it does not induce pollen sterility. At the same time, the frequency of spontaneous breaks is also considerably reduced in the case of $2n$ *tabacum*. It is assumed that the *debneyi* genome confers an instability on the *longiflora* chromosome, and the pollen sterility segment is "activated" by the *debneyi-tabacum* genome and is "inactive" in the $2n$ *tabacum* genome.

In the cross $4n$ *N. debneyi-tabacum* $\times$ *N. longiflora* there is an effect on pollen abortion when a particular *longiflora* chromosome is present. A similar effect of an alien chromosome was previously reported in a cross involving different species of *Nicotiana*. CAMERON and MOAV (1957) reported that diploid, sesquidiploid and amphidiploid hybrids involving *Nicotiana tabacum* $\times$ *N. plumb-*

ginifolia were male sterile but that the latter two were female fertile. Repeated backcrossing of the sesquidiploid to diploid *tabacum* accompanied by selection for a dominant *plumbaginifolia* genetic marker resulted in the establishment of 24tt + 1p types. They determined that in addition to the locus for disease resistance, the chromosome from *plumbaginifolia* carried a locus for "pollen killer." The latter has no deleterious effect on the pollen of *N. plumbaginifolia* itself but in 24tt + 1p derivatives it caused abortion of 24t pollen shortly after meiosis at the microspore stage. There appeared to be no adverse effect of "pollen killer" on megaspores or somatic cells.

SUMMARY

A number of *Nicotiana* species hybrids, diploid as well as multiple genomic, were made in order to study the cytogenetics of genetic tumors formed in certain interspecific combinations. These tumors are spontaneously produced on stems, roots and occasionally on leaves of all the F_1 progeny or only a part of it.

The cross $4n$ *N. debneyi-tabacum* $\times$ *N. longiflora* gives tumors in all the F_1 progeny. By repeated backcrossing to $4n$ *debneyi-tabacum* for three generations accompanied by selection for tumor production, it was found that in these backcross plants an alien *longiflora* chromosome on a *debneyi-tabacum* background causes tumor production. A similar cytogenetic analysis of a backcross progeny of $4n$ *N. longiflora-tabacum* $\times$ *N. tabacum* for two generations showed that probably here too a specific *longiflora* chromosome is involved in tumor production. These data differ from those reported in other hybrid combinations by KEHR and SMITH (1954) and SMITH and STEVENSON (1961).

On the basis of morphology, four classes of plants may be recognized. Class I contains tumorous and pollen-sterile plants. Class II consists of tumorous and pollen-fertile plants. Class III is composed of nontumorous and pollen-sterile plants. Class IV is composed of normal plants.

A high frequency of spontaneous fragments was observed in *longiflora* chromosomes in otherwise *debneyi-tabacum* plants. The presumptive cytological picture in the four classes was as follows. Class I plants carried the *longiflora* chromosome with the tumor and pollen-sterility inducing regions. Class II contained a centric fragment of the alien chromosome presumably carrying the tumor causing segment and the fragment associated with pollen-sterility is assumed to be lost. Class III plants also contained a centric fragment but presumably with the tumor-inducing region eliminated. A few plants in class IV have a centric fragment of the *longiflora* chromosome supposedly with neither of the two regions under study. However, most of the plants in this class did not carry any portion of the alien chromosome.

A 2×2 contingency test of the four classes showed a definite relationship between tumor production and pollen sterility, and supported the assumption that both factors are located on the same chromosome. The ovular transmission of the *longiflora* chromosome was about 40 percent in $4n$ *N. debneyi-tabacum* $\times$ *N. longiflora* hybrids.

Grafting experiments between tumorous and normal plants and application of tumor extract to the cut surfaces of leaves and stems of normal plants gave no indication of the presence of a transmissible agent.

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