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CYTOGENETIC STUDIES IN DERIVATIVES OF NICOTIANA RUSTICA × N. TABACUM

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INTRODUCTION

Nicotiana tabacum and *N. rustica* have each several characters which can supplement the needs of the other. *N. rustica* is early, requiring only 45 to 60 days to flower, has a higher nicotine content and is resistant to several diseases like powdery mildew, wild fire and blue mould, besides possessing field resistance to mosaic. *N. tabacum* has a higher leaf number, larger leaf size and a wider adaptability to growing conditions. The cross between these two species has been found to succeed only when *N. rustica* is used as the pistillate parent, though seed setting is poor owing to the abortion of immature embryos (BRINK and COOPER, 1). The reciprocal cross normally fails due to the inadequate growth of *rustica* pollen tubes in *tabacum* style (SWAMINATHAN and RADHAKRISHNA MURTY, 14). Hybrids from the cross *N. rustica* × *N. tabacum* have been studied by several authors (KOSTOFF, 7; GOODSPEED, 6). SMITH (9, 10, 11) investigated in detail the cytology of *rustica-tabacum* hybrids and has backcrossed the F_1 plants several times to *N. rustica*. In this way he transferred the recessive "mammoth" gene of *N. tabacum* (which causes the otherwise day-neutral plants to flower only under short photoperiod) to *N. rustica* in which this mutation has never been found to occur spontaneously.

Plants belonging to the second segregating generation of the sixth backcross of F_1 (*N. rustica* × *N. tabacum*) to *N. rustica* raised from seeds kindly provided by Dr. HAROLD H. SMITH of the Cornell University, were grown in Delhi during the years 1955 and 1956 in order to compare their performance with the improved *rustica* strains produced at this Institute. These plants were found to possess several characters of *N. tabacum* and to show some floral abnormalities as well as meiotic aberrations, seed sterility and a peculiar behaviour with reference to the expression of mammoth character. In the present report, some of the observations made in this material are recorded.

MATERIAL AND METHODS

Two families of the *rustica-tabacum* material were received from Dr. SMITH (Nos. 55379 and 55380). Part of the material was grown in a glasshouse and the rest was planted in the field. Field plantings were done in January and February. For purposes of comparison, N.P. 219, an improved strain evolved at the Indian Agricultural Research Institute, was grown both in the field and in the glasshouse. Purpurea, the *tabacum* parent used in the cross was also grown in the glasshouse. The hybrid seeds were shrivelled and varied in colour from dark to pale brown. Germination was less than 40 per cent, in contrast to the more than 96 per cent germination shown by normal *rustica*.

For the study of meiosis in microsporocytes, anthers were fixed in carnoy's fluid (6:3:1) for 24 hours and squashed according to the propiono-carmine schedule (SWAMINATHAN *et al*, 13). Nicotine content in leaf samples was estimated spectrophotometrically (RAMAMURTY *et al*, 8).

OBSERVATIONS

Growth habit and yield: The hybrids were more-vigorous than *N. rustica* (N.P. 219) throughout the period of growth (Figs. 1 and 2). There was a pronounced suckering and branching-tendency in the hybrids. Statistical analysis of the data (Tables 1 and 2).

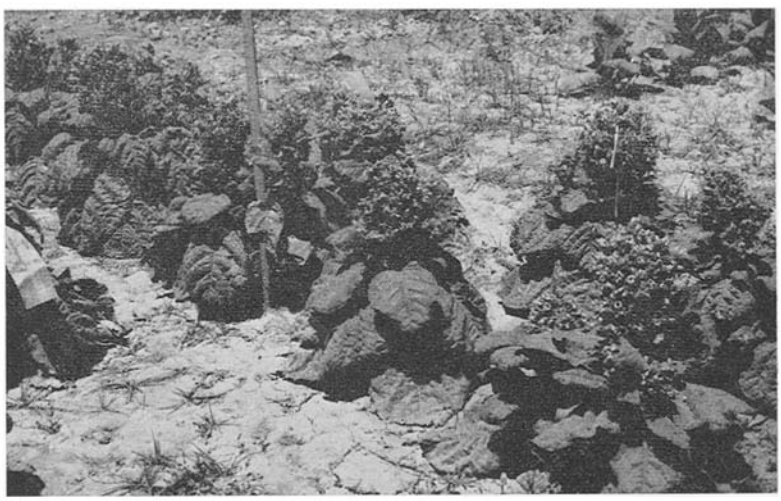

FIG. 1. PLANTS OF *N. rustica* (N.P. 219) GROWING IN THE FIELD

FIG. 2. PLANTS OF *rustica-tabacum* GROWING IN THE FIELD

TABLE 1. YIELD AND GROWTH CHARACTERS OF *rustica-tabacum* HYBRIDS

Material	No. of plants	No. of days to flower	Range	Height in cms.	Panicle length cms.	No. of leaves per plant	Largest leaf		Mature capsules/ plant	Yield in gms/ plant	Nicotine content (%)
							L. cms.	B. cms.			
N.P. 219	10	54.50	48–61	56.61	27.12	11.80	23.40	24.31	112.82	65.82	2.88
55379											
(Early planting)	7	89.57	80–99	28.59	53.41	23.29	26.93	20.40	0	145.70	2.72
[1] (Late planting)	3	–		89.80	38.67	17.33	21.17	17.53	0	–	–
55380											
(Early planting)	16	75.80	62–92	117.29	49.11	20.25	24.18	20.54	21.69	134.98	2.38
[1] (Late planting)	9	–		80.76	33.78	17.00	22.19	17.43	0	–	–
Purpurea	1	89.00	122.60	46.20	29.00	32.50	14.20	138.00		–	–

[1] The number of days to flower and yield data were not recorded.

TABLE 2. PLANT GROWTH MEASUREMENTS OF *rustica-tabacum* HYBRIDS EXPRESSED AS PERCENTAGE OF THOSE OF *N. rustica*

Material	Days to flower	Height	No. of leaves	Leaf size		Yield	No. of mature capsules
				L.	B.		
A. N.P. 219	100	100	100	100	100	100	100
B. 55379	164.4	229.2	197.4	115.1	83.9	221.4	0
C. 55380	139.4	207.2	171.6	103.3	84.5	105.1	19.24
Significance by 't' test	$\overline{B}\,\underline{C}\,\overline{A}$	$\overline{B\,C}\,\underline{A}$	$\overline{B\,C}\,\underline{A}$	$\overline{A\,B\,C}$	$\underline{A}\,\overline{C\,B}$	$\underline{B}\,\overline{C}\,\underline{A}$	$\overline{A}\,\underline{B}\,\overline{C}$

revealed significant differences in height, time of flowering, number of leaves per plant, yield of dry leaf and number of mature capsules per plant between the hybrids and *N. rustica*. There was no significant difference in leaf size. It is interesting to state that the hybrids had twice the height of *N. rustica*, although the proportion of the size of panicle to plant height was similar in both. Flowering was delayed in the hybrids by 20 to 35 days and it also varied much in the different hybrid plants. One of the hybrid families (55379) took 15 days more than the other to flower. The increased vegetative growth in plants of this family is probably responsible for the increased yield obtained from them.

When the characters of the hybrids were compared with those of *N. tabacum* var. *Purpurea* it was observed that plant height, leaf shape and number, panicle type and the expression of mammoth character are some of the traits transferred from *N. tabacum* to the hybrids (Figs. 2, 3 and 4).

Floral abnormalities: All the hybrid plants had some flowers with split corolla, an abnormality not seen in *N. rustica*. The proportion of such flowers to normal flowers in the different plants ranged from 12 to 85 per cent. In two plants the flowers had a vestigeal corolla, very short styles (2 to 3 mm. in length), mutilated stigmas and a calyx fused with the style (Fig. 5). The anthers in such flowers were rudimentary and did not contain any pollen. No seed setting was obtained even in pollinations with normal pollen. These plants were found to show partial asynapsis.

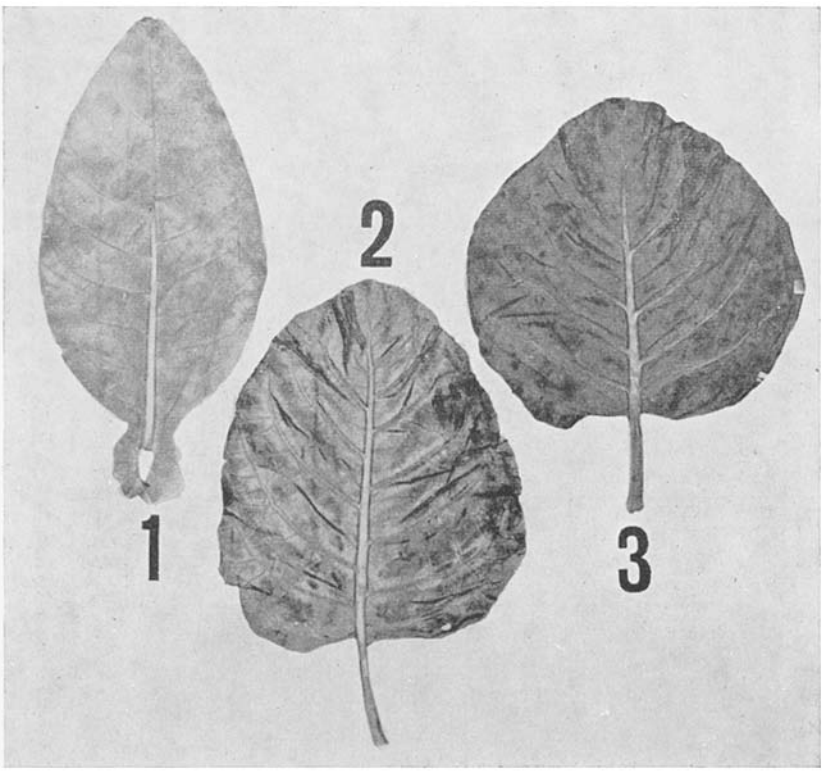

FIG. 3. LEAVES OF (1) *N. tabacum* VAR. PURPUREA, (2) *rustica-tabacum* HYBRID AND (3) *N. rustica*

Segregation for mammoth character: As already mentioned, SMITH (11) transferred this character from the variety Maryland Mammoth of *N. tabacum* to *N. rustica* in order to increase the leaf number in the latter species. SMITH also found that this character is controlled by a single recessive gene. During the present study 29 hybrid plants had been kept in the glasshouse and 23 had been planted in the field. The sowing had been done at the same time in both cases. Among the plants grown in the glasshouse, 6 were found to be mammoths (2 in culture No. 55379 and 4 in culture No. 55380). No mammoth plants, however, occurred in the field. Theoretically, the cultures may be expected to segregate in the ratio 3 normal: 1 mammoth if they were initially heterozygous for the locus. It was hence interesting that while both the cultures segregated for this character in the glasshouse, all those that were grown in the field behaved as normal plants. That the plants classified as mammoths were not just normal plants in which flowering was delayed, was shown by the fact that some of the plants from the same material sown as late as the last week of March flowered in June.

Cytological observations: Microsporogenesis was studied in 23 plants. Six plants were found to be asynaptic and meiosis was characterised by regular bivalent formation in the rest. The data are given in Tables 3, 4 and 5. While diakinesis was usually normal with 24 bivalents, the chromosomes at metaphase were sticky (Figs. 6 and 7).

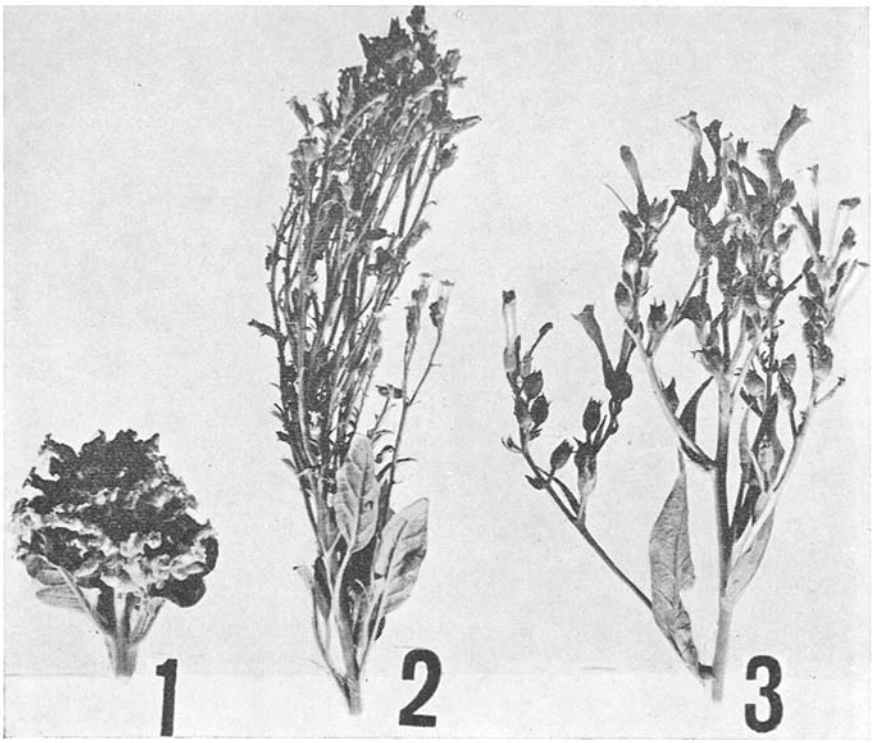

FIG. 4. PANICLES OF (1) *N. rustica* (2) *rustica-tabacum* HYBRID AND (3) PURPUREA

Anaphase irregularities included sticky bridges, bridges without fragments, dicentric bridges and acentric fragments and lagging chromosomes ranging from 1 to 11 in number. There was unequal distribution of chromosomes in second metaphase plates. There were occasional bridges and lagging chromosomes at second anaphase. Besides normal tetrads, monads, dyads, triads and polyads as well as many micronuclei were observed at the sporad stage (Table 5, Fig. 8). Pollen was of variable sizes. Some were multi-nucleate with a maximum number of six nuclei. While normal pollen measured 38.1 × 41.9 microns, some large grains measured 50.8 × 54.5 microns. A few dyads were also found to be sticking together while the other spores were free (Fig. 9).

Pollen and seed fertility: In proportion to their size, the anthers contained very little pollen. Pollen fertility as tested by stainability in acetocarmine, was found to vary much and ranged from 0 to 71 per cent in the different plants. Those with complete pollen sterility were later identified as asynaptic plants. The number of capsules per plant varied from 0 to 73 in the hybrids, while *N. rustica* plants had on an average 113 capsules per plant. There were about 250 seeds per capsule in *N. rustica* in comparison with the 0 to 132 seeds per capsule observed in the hybrids. Plants which commenced flowering in the last week of April did not show any seed setting even when hand-pollinated. *N. rustica* plants, however, set some seeds under hand pollination during the hot weather.

TABLE 3. CHIASMA FREQUENCY AT DIAKINESIS IN *N. rustica*, *N. tabacum* AND *rustica-tabacum*

Material	No. of PMCs	Configurations with xta				xta per PMC	Range of xta
		0	1	2	3		
N. tabacum var. *Purpurea*	10	0.33	5.75	17.67	0.41	42.33	40–44
N. tabacum var. *Natu*	20	0.00	3.10	18.55	2.35	47.25	46–49
N. rustica	15	0.00	4.20	16.73	3.07	46.87	45–49
55379 (Normal plants)	45	0.27	1.18	19.17	3.55	50.07	49–50
55380 (Normal plants)	30	0.61	1.98	19.33	2.32	48.86	48–49

TABLE 4. MEIOSIS IN NORMAL PLANTS OF RUSTICA-TABACUM

Family and plant No.	Metaphase I		Anaphase I		Anaphase II		Balanced gametes percent
	No. of PMCs	Percent abnormal	No. of PMCs	Percent abnormal	No. of PMCs	Percent abnormal	
55379							
28/1	40	95.0	63	25.4	105	85.7	60.0
[1] 28/2	70	92.9	61	44.3	57	33.3	–
[2] 4/2	111	36.0					
55380							
22/1	91	87.9	65	90.8	53	67.9	62.3
30/1	58	17.2	56	21.4	59	25.4	80.0
5/5	44	86.4	98	33.7	52	34.6	77.8

TABLE 5. SPORAD ANALYSIS, POLLEN AND SEED FERTILITY AND OTHER CHARACTERS OF NORMAL PLANTS OF RUSTICA-TABACUM

Family and plant No.	No. of PMCs	Normal Tetrads %	Pollen Fertility %	No. of capsules/plant	Seeds per capsule	Dry leaf yield in gms
55379						
28/1	92	75.0	19.9	54	100.0	98.8
28/2	99	68.7	22.4	76	30.1	101.5
[1] 4/2	–	–	4.0	3	0	159.1
55380						
22/1	96	36.5	48.6	65	38.3	79.8
30/1	118	69.5	70.3	73	78.2	81.3
5/5	76	59.2	19.7	8	90.6	141.2

[1] Not analysed for sporads

Asynaptic plants: Three plants in culture No. 55380 were found to be completely asynaptic, chromosome pairing failing to take place from pachytene onwards. In such plants, a maximum frequency of 4 bivalents could be seen in some cells. All the bivalents were loosely associated with chiasma in only one of the arms. Sticky bridges, lagging univalents and unequal distribution of chromosomes were observed at anaphase I and II. Pollen sterility was nearly complete, though occasionally a few large well stained pollen kernels probably representing dyads or monads, were seen.

In addition to the completely asynaptic plants, there were three partially asynaptic plants in which there were on an average 16.92 bivalents and 14.16 univalents per cell. The maximum number of bivalents observed was 18. The partially asynaptic plants also had various other meiotic abnormalities like irregular disjunction, stickiness, formation of micronuclei at the sporad stage etc. Pollen fertility was low, only 11.6 per cent of pollen being stainable.

The number of mature capsules varied from plant to plant. Under conditions of open pollination, two completely asynaptic plants which flowered 17 to 24 days earlier than the third, had 34 and 56 capsules with 71.1 and 35.1 seeds per capsule respectively. The third plant which flowered 88 days after planting did not form any capsule. A partially asynaptic plant had 73 capsules with 131.8 seeds per capsule.

Discussion

The *rustica – tabacum* plants studied by us were derivatives of the sixth backcross to *rustica*. They still resembled *tabacum* in leaf shape, leaf number, profuse suckering, panicle type and duration of flowering. The question arises as to what this persistence of *tabacum* – characters, in spite of repeated backcrossing, is due. In the F_1 *N. rustica* × *N. tabacum* up to 7 bivalents have been reported to occur (Goodspeed, 6). This frequency is much higher than what is found in haploids of either *N. tabacum* or *N. rustica*. In fact, haploid plants of Purpurea, the *tabacum* variety used in the cross, show only one bivalent per cell and that too rarely (Goodspeed, *l.c.*). It is thus clear that some chromosomes of *rustica* and *tabacum* pair with each other at the F_1 stage. Eghis (5) regards the transfer of characters from *tabacum* to *rustica* as being due to an interchange of small segments between the chromosomes which pair in the F_1. Even whole chromosomes could be involved in such a transfer, since, according to Clausen (2), introgression of characters in several species hybrids takes place in this manner. In the hybrid plants studied during the present investigation there was no reduction in chiasma frequency in comparison with the values found in *tabacum* and *rustica*. There were, however, several meiotic abnormalities chiefly of a physiological nature but probably genetically controlled and also a considerable reduction in seed setting in comparison with *N. rustica*. Since the material has been selfed before being backcrossed in several stages (Smith, 11), it is possible that there are some chromosome segments of *tabacum* translocated into *rustica* chromosomes. Such differential segments would behave as units in inheritance and would tend to be passed on unaffected to the various backcross generations. If only some individual loci instead of chromosome segments are involved, there would be segregation for the characters in the different progenies and with every backcross to *rustica* there would be a gradual elimination of the *tabacum* genes unless conscious selection for such characters is practised. The existence of cryptic structural differences in the chromosomes of the *rustica – tabacum* hybrids with the consequent deficiencies and duplications caused in the gametic chromosome complements, would therefore explain most of the observed results.

Mammoth character: The genetics of several characters has been followed in the cross *N. rustica* × *N. tabacum* and Eghis (5) found simple monogenic segregation for some of them. However, the inheritance of the Mammoth gene, which has been identified only in *N. tabacum*, is the best worked out among the various genetic studies

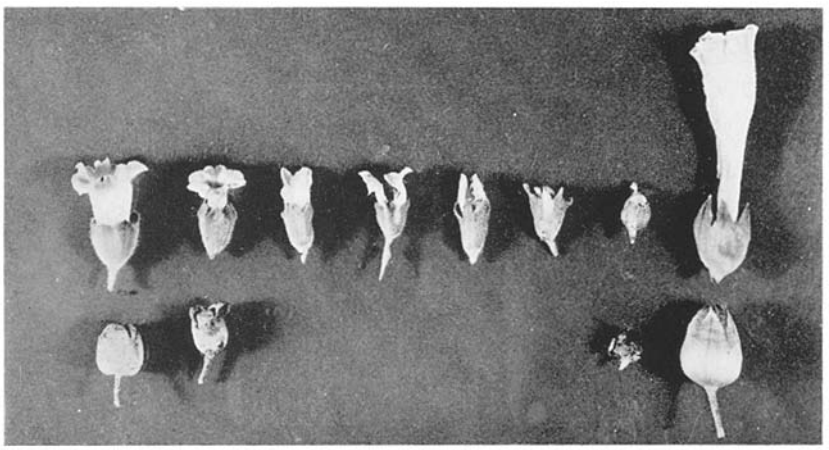

FIG. 5. FLOWER ABNORMALITIES IN *rustica-tabacum* HYBRIDS. FLOWERS OF *rustica* AND *tabacum* ARE ON THE LEFT AND RIGHT EXTREMES RESPECTIVELY

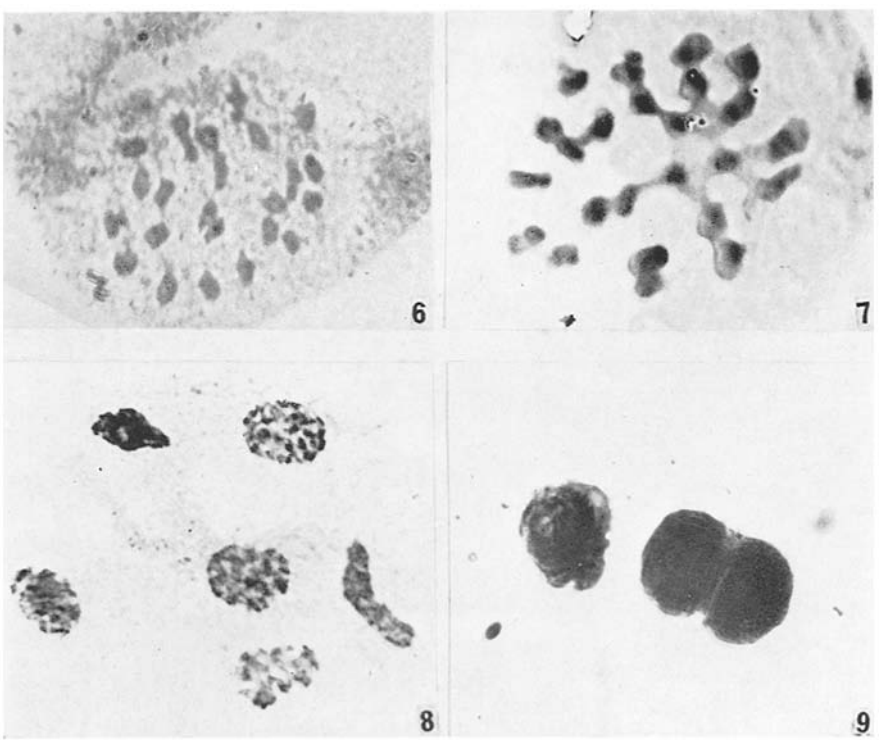

FIG. 6 TO 8. MICROSPOROGENESIS IN A *rustica-tabacum* HYBRID

FIG. 6. METAPHASE I SHOWING 24 BIVALENTS

FIG. 7. METAPHASE I SHOWING STICKINESS

FIG. 8. TELOPHASE II WITH 6 GROUPS OF CHROMOSOMES

FIG. 9. SOME LARGE POLLEN KERNELS OF *rustica-tabacum* (ONE IS A DYAD)

in *rustica* – *tabacum* hybrids. In *N. tabacum*, it has been found to be inherited as a simple recessive. The fact that from the 3rd and 4th backcross generations the segregations were simple, made SMITH (11) conclude that a cross-over rather than substitution or addition of a *tabacum*-chromosome has caused the transference. During the present study, segregation for what appears to be mammoth character occurred only in plants grown in pots (out of 29 plants, 6 were mammoth). The segregation in this case could be explained on a simple Mendelian basis. However, there was no segregation for mammoth character in plants grown in the field. SMITH (9) who obtained segregation for mammoth in only one out of 5 different crosses with *rustica-tabacum* plants, explained this behaviour on the assumption that the chromosomes carrying the mammoth gene were lost in the plants segregating for mammoth. Such an explanation cannot be applied to the situation described in this paper. Obviously the expression of the mammoth character is an inter-related response of heredity and environment. SMITH (11) failed to make the mammoth plants flower under 6, 8 and 18 hour day lengths. Owing to the failure of *rustica-tabacum* mammoths to flower, SMITH could not study the meiosis in such plants. In this connection, some interesting results obtained by STEINBERG (12) give a possible explanation for mammoth segregants occuring in the glasshouse and being absent under field conditions.

STEINBERG (12) found that *rustica-tabacum* mammoths did not flower when grown under warm conditions (75 °F) but flowered when cultivated under lower temperatures (i.e. 65 to 75 °F during day; 50 to 60 °F during night). Thus, the mammoth gene, transferred from *N. tabacum*, no longer behaves as a day-length preceptor but functions as a temperature – flowering preceptor. The temperature conditions at Delhi during January-February in the field closely approximate the requirements mentioned by STEINBERG as necessary for mammoths to flower. This could have caused flowering in them, while under glasshouse conditions the day temperatures were higher and the fluctuations between day and night temperatures were not as great as in the field. This might be responsible for the mammoth plants not commencing to flower at all. STEINBERG has further stated that day-neutral *tabacums* may also respond to a low temperature treatment for the stimulation of flowering. In fact, Nattu, a local variety of *N. tabacum*, was found to behave at Delhi in a way which suggested the need of cold temperatures for flowering. The plant suddenly ceased its reproductive growth and started growing again vegetatively when the temperature rose from April onwards. All these facts tend to support the view expressed by DOBZHANSKY and HOLZ (4) that "Genes produce not characters but physiological states which through interactions with the environmental influences, cause the development to assume a definite course and the individual to display certain characters at a given stage of the development process".

Another interesting aspect of the flowering response of the *rustica-tabacum* mammoth relates to SMITH'S (11) observation that with each successive backcross to *rustica* there was a decrease in the number of mammoth plants which flowered under short day conditions. An analogous behaviour is the increase in male sterility observed by CLAYTON (3) in successive backcrosses of *N. debneyi* × *N. tabacum* to *tabacum*. As in the *debneyi* cross, cytoplasm – nucleus interaction may play an important role in controlling the response of the mammoth gene in a *rustica* background. It is significant in this connection that mammoth character, though of frequent occurrence

in *tabacum*, is absent in *rustica* and is gradually suppressed in hybrid derivatives involving *rustica*. Even in the postulated progenitors of *rustica*, mammoth gene has not been recorded.

Economic importance of the rustica-tabacum derivatives

The hybrid derivatives had many of the desirable qualities of both parents. Also, without reduction in the nicotine content, they gave a higher yield in comparison with *rustica*. Late flowering and reduction in seed setting are the important handicaps. Since there was a considerable variability in the manifestation of these characters in individual plants, it may be possible to subject the population to further rigorous selection. The population was fairly uniform with reference to plant habit and vigour and consequently selection for earliness and better seed setting may not affect the yield and leaf characters.

SUMMARY

Two families belonging to the second segregating generation of the sixth backcross of (F_1 *N. rustica* × *N. tabacum*) to *N. rustica* were studied and it was found that both populations were cytologically unstable. However, the plants were uniform in most of the morphological characters.

Segregation for "mammoth" character occurred only in plants kept in the glass-house. It is suggested that the "mammoth" gene behaves as a temperature preceptor for flowering in a *N. rustica* genotype.

The hybrids resembled *N. tabacum* in height, leaf number, leaf shape and panicle type and this persistence of the characters of *N. tabacum*, in spite of repeated back-crossing to *N. rustica*, is explained on the assumption that small segments of *tabacum*-chromosomes have been translocated into those of *rustica*. Such a type of cryptic structural differentiation of chromosomes in the hybrids would explain many of the observed phenomena like abnormalities in meiosis, seed sterility and block transference of *tabacum*-characters.

From an economic point of view, the hybrids are superior to *rustica* in yield and it will be possible to evolve from them commercial strains by further selection for earliness and fertility.

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SAMENVATTING

Cyto-genetische onderzoekingen van materiaal afstammend van Nicotiana rustica × N. tabacum

Twee families behorende tot de 2e splitsende generatie van de 6e terugkruising van de F_1 *N. rustica* × *N. tabacum* met *N. rustica* werden onderzocht. Beide populaties bleken cytologisch niet stabiel te zijn, doch waren uniform voor de meeste morfologische kenmerken.

Splitsing in normaal en "mammoth" (recessief gen voor korte dag) werd alleen waargenomen bij planten in het warenhuis. Verondersteld wordt dat het mammoth-gen sterk beinvloed wordt door de temperatuur.

De bastaarden geleken op *N. tabacum* in hoogte, aantal bladen, bladvorm en bloeiwijze. Verondersteld wordt dat het voorkomen van *N. tabacum*-eigenschappen, niettegenstaande herhaalde terugkruising met *N. rustica*, berust op vervanging van kleine segmenten van de *tabacum* chromosomen door die van *rustica*. Met deze structuurveranderingen van de chromosomen zijn vele van de waargenomen verschijnselen (abnormale meiosis, steriliteit, koppeling van *tabacum* eigenschappen) te verklaren.

De bastaarden zijn productiever dan *rustica*, zodat verwacht wordt dat bij voortgezette selectie op vroegheid en fertiliteit praktijkrassen verkregen zullen worden.

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