INHERITANCE IN *NICOTIANA TABACUM*

XXX: AUTOTRIPLOIDY, A POSSIBLE MEANS OF INCREASING THE RATE OF INTERSPECIFIC GENE TRANSFER

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1. INTRODUCTION

THE smaller the degree of effective homology between chromosome sets of two different species, the lower the frequency of interspecific chromosomal pairing and segmental exchange in their hybrids (Clausen, 1952). This low rate of exchange is a limiting factor restricting utilisation of interspecific gene transfer in experimental and commercial breeding of plants. A search for methods which would increase the rate of exchange has led (on theoretical grounds) to the hypothesis that autotriploidy of a recipient species should provide alien chromosomes with a better chance for pairing and exchange of segments with the recipient's chromosomes.

The present paper describes an attempt to test this hypothesis experimentally with hybrid derivatives of *Nicotiana tabacum* and *N. plumbaginifolia*.

2. MATERIALS AND METHODS

The "White-Seedling" (*Ws*) locus of *Nicotiana tabacum* ($n = 24$) was chosen (Moav, 1957). Its recessive allele *ws* conditions an inability to induce chlorophyll production. The dominant gene of the species *N. plumbaginifolia* ($n = 10$), which covers the effect of *tabacum*'s *ws* has been designated *Ws(pbg)*.

When introduced by hybridisation into a *tabacum* nucleus, the *plumbaginifolia* chromosomes become somatically unstable, that is, they tend to be lost during somatic divisions. Thus hybrids carrying the recessive *ws* alleles in all their *tabacum* genomes are usually mottled (Ar-rushdi, 1957; Cameron and Moav, 1957; Moav, 1958; Moav, 1961). This character provides an easy and fairly reliable phenotypic distinction between hybrid derivatives which carry only *ws* alleles (mottled) and those possessing one or more *Ws* alleles (self green) in their *tabacum* genomes.

3. EXPERIMENTAL PROCEDURES AND RESULTS

The immediate purpose of the experiments was a comparison of the rate of transfer of the *Ws(pbg)* locus to *N. tabacum* chromosomes in autotriploid and diploid nuclei. The tests on the diploid level were carried out with mottled diploid *heterozygous alien addition* plants (2 sets of *tabacum* chromosomes plus a *plumbaginifolia* univalent carrying the *Ws(pbg)* locus: $2n \text{ } ws \text{ } ws(tbc) + 1 \text{ } Ws(pbg)$). The equivalent autotriploid derivatives ($3n \text{ } ws \text{ } ws \text{ } ws(tbc) + 1 \text{ } Ws(pbg)$) were selected from

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progenies of crosses between simplex autotetraploid *tbc* ($4n\ Ws\ ws\ ws\ ws(tbc)$) with the mottled diploid alien addition plants ($2n\ ws\ ws(tbc) + 1\ Ws(pbg)$). The desirable alien addition triploid offspring were mottled. They were progeny tested to determine the unpaired condition of the *plumbaginifolia* (henceforth abbreviated *pbg*) univalent (table 1).

TABLE 1

Transmission rates of the *Ws(pbg)* locus of the mottled autotriploid alien addition plants ($3n\ tbc\ ws\ ws\ ws + 1\ Ws(pbg)$), when backcrossed to diploid homozygous recessive *tabacum* ($2n\ tbc\ ws\ ws$) *

Tested autotriploid plants	Proportion of non-albino offspring †	Designation of back-cross populations
	Per cent.	
55R91p2	24·8	65R55, 56R56 ‡
55R92p2	24·2	56R57
55R100p1	20·5	56R62
56R01p2	20·3	56R85

* Albino (chlorophyll-less) shoots that developed on mottled diploid alien addition plants served as homozygous recessive parents.
† The proportion of non-albino (self-green plus mottled) offspring is equal to the transmission rate of the *Ws(pbg)* locus. Over 100 seedlings were tested in each case.
‡ The two populations originated from two different capsules.

FIG. 1.—THE BREEDING PROCEDURE.
(for details see text)

Four tested autotriploid alien addition plants were backcrossed to homozygous recessive diploid *N. tabacum* (henceforth abbreviated *tbc*), and five samples of offspring each of 26 randomly selected non-albino seedlings were grown and progeny tested (table 1, right column). The breeding procedure is presented schematically in fig. 1.

Plate

FIG. 1.—Some ears obtained from self-pollination of $De^{t1}de^{t2}/de^{t1}De^{t2}$ plants. The majority of plants of this constitution give rise to ears showing about 50 per cent. of defectives, together with a minority of ears segregating for only one of the two defective types, for both defectives in coupling phase and very rare cases of ears with no defective. The ratio of such four types of ears from the normal viable seeds of these ears has been found to be about 68 : 20 : 2 : 1, indicating the existence of a balanced lethal system, with about 14 per cent. of recombination between the two defective factors.

After self-pollination, 94 plants produced sufficient amounts of seeds for progeny tests. Of the remaining, 18 yielded abundant seed when pollinated with pollen of diploid homozygous recessive plants (table 2). One plant (56R55p24, table 2) did not bear seeds,

TABLE 2

Transmission rates of the Ws(pbg) locus in the tested and control plants
(see figure 1, and text) *

Plant No.	Popula- tion No.	Per cent. of non-albino offsprings (<i>Ws(pbg)</i> transmission)						
		56R55	56R56	56R57	56R62	56R85	56R09†	56R16†
1		54.5	20.0	20.0	41.8	47.6	11.6	6.7
2		25.0	21.3	...	18.2	...	15.6	15.4
3		33.2	23.1	42.9	16.3	9.8	10.9	...
4		48.9	10.6+	19.3+	16.7	22.4	18.1	8.4
5		8.1	8.0+	...	31.9	25.0+	22.8	18.7
6		18.6+	24.5	14.9+	10.1	20.3+	10.4	11.9
7		11.4	13.9	13.2	24.0	28.0	11.7	17.4
8		39.6+	26.2	26.9+	7.1+	13.0	9.4	17.1
9		60.0	21.8	17.5	3.7	8.5	15.4	22.9
10		23.9	11.9+	27.5	12.2	18.4	18.6	11.1
11		42.7	32.4	...	21.4	22.6+	18.8	25.7
12		42.0	35.5	...	24.9	12.7	10.8	17.0
13		17.6	24.6	14.3	18.7	21.7	16.0	53.3
14		21.6	23.8	15.2	34.1+	24.8	9.7	11.6
15		17.9	18.7	55.2	20.9	29.2	12.8	25.0
16		22.7	24.6	42.0	14.0	29.4	17.0	13.2
17		20.0	9.6	37.8	28.9	17.9	8.9	11.4
18		21.1	9.0+	10.3	38.0	...	10.2	11.1
19		22.3	17.2	27.0	23.6	...	14.9	15.5
20		24.0	22.3	31.9	...	...	18.8	9.0
21		19.6	20.4	31.5	15.2+	...	20.2	16.9
22		48.4	28.6	12.5	26.5	16.5	14.6	15.9
23		21.9	15.1	28.5	45.1	13.0	22.6	8.9
24		60.2+	28.5+	23.8+	...	...	24.3	8.0
25		44.6	36.9	...	11.4	...	...	18.7
26		...	26.5	...	9.5+	...	...	17.2
No. above 32.5 per cent.		10	2	4	4	1	0	1
Per cent. no. above 32.5 per cent.‡		9.9	1.9	4.8	3.4	1.1	0	0.7

* Many of the values in the body of the table are averages of several samples each taken from a different capsule. In some of the progenies the inter-capsule intra-plant variability was much higher than expected. In each progeny over 100 seedlings were germinated. The numbers followed by a + are the results of backcrosses to the diploid homozygous recessives. The rest are products of selfing.

† Diploid control ($2n\ ws\ ws + 1\ Ws(pbg) \times self$).

‡ Percentages of tested plants, including albino seedlings, that transmitted the *Ws(pbg)* locus to more than 32.5 per cent. of their offspring. These percentages are estimates of the rate of interspecific chromosomal exchanges which have transferred the *Ws(pbg)* locus to a *tbc* chromosome in the parents of the tested plants.

either on selfing or when pollinated with pollen of diploid *tbc* plants, but its own pollen proved to be functional when back-crossed to diploid females. Both selfing and back-crossing of the remaining 17 plants failed to yield functional seeds.

For control, two samples of selfed mottled diploid heterozygous alien addition plants ($2n\ ws\ ws(tbc) + 1\ Ws(pbg)$) were progeny tested (table 2, the two right columns). In fig. 2, the frequency distribution of the "transmission rates" of all the five autotriploid samples are compared with the equivalent distribution of the diploid controls.

Division of the abscissa at the 32.5 per cent. point, produced two classes: (i) Up to 32.5 per cent. transmission of the $Ws(pbg)$ locus. Most of the plants that fell into this class presumably carried the $Ws(pbg)$ locus on the original unpaired pbg univalent. (ii) Above

FIG. 2.—Distribution of the transmission rates of table 2.
Upper histogram. Diploid control ($2n\ ws\ ws + 1\ Ws(pbg) \times \text{self}$), 49 plants.
Lower histogram. Self progeny of Autotriploid $\times$ diploid parents ($3n\ ws\ ws\ ws + 1\ Ws(pbg) \times 2n\ ws\ ws$) 94 plants.

32.5 per cent. transmission. The high transmission of this class is chiefly due to attachment of the pbg segments carrying the $Ws(pbg)$ locus, to tbc chromosomes. The interspecific chromosomal exchanges (rare crossing-overs or translocations) which are responsible for the above attachments have taken place (with rare exceptions) during meiosis of the tested alien addition parents of the tested plants. Thus, the proportion of plants which fell into the second class provided estimates for the rate of chromosomal exchanges between the alien $Ws(pbg)$ carrying chromosome and the tbc genome. The estimates are given in the bottom line of table 2.

The choice of 32.5 per cent. for a boundary, probably furnished low estimates for the rate of exchanges. It is most likely, that the majority of 27.5-32.5 per cent. transmission group were also products of chromosomal interchanges of long *pbg* segments or interchanged chromosomes in a "trisomic" condition, where the interchanged chromosome has two semi-homologous unmodified *tbc* chromosomes.

The proportion of the "above 32.5 per cent." in all the autotriploid back-cross progenies was higher than that of the two control samples. Similar experiments with the same locus were previously conducted on a large scale at the diploid level (Moav, 1958, 1961). These experiments indicated an interspecific chromosomal exchange frequency of 1 to 1.5 per cent. Fitting the observed rates of the autotriploid samples to 1.5 per cent. and using the binomial test, showed a significant difference.

4. DISCUSSION

First metaphase meiotic plates of pollen mother cells of F_1 hybrids between *Nicotiana tabacum* (*tbc*) and *plumbaginifolia* (*pbg*), show a mode of 3 bivalents (Goodspeed, 1954). The bivalents are held by single chiasmata and are visibly heteromorphic. Since *tbc* haploids display an average of one bivalent, it may be assumed that the average interspecific meiotic pairing in the F_1 hybrids is only two bivalents per sporocyte (Cameron and Moav, 1957). Thus, the average bivalent formation for a single *pbg* chromosome ($n = 10$) should be 0.2 per sporocyte, or one bivalent in five sporocytes. If it is further assumed that the observed bivalents are held by true chiasmata that result in exchange of chromosomal segments, then an average of 10 per cent. of the hybrid's spores should carry a *pbg* marker on exchanged chromosomes. The F_1 *tbc-pbg* hybrids are completely sterile but the sesquidiploids ($2n\ tbc + n\ pbg$) and their back-crosses to *tbc* are fertile. In the last group the above rate of interspecific chromosomal exchange was actually measured with several loci and was found to be much lower than the expected 10 per cent. The *Ws(pbg)* marker was incorporated into a *tbc* chromosome in around 1 per cent. of the offspring, and the proportion of other *pbg* loci which were studied were even lower (Moav, 1958; Moav and Cameron, 1960).

The best explanation for the discrepancy between the expected and observed rates of interspecific chromosomal exchanges seems to be *competition for pairing*. That is, in sesquidiploids and diploid alien addition derivatives, each *tbc* chromosome has a completely homologous chromosome to pair with, therefore it would only rarely pair with the alien chromosome with which it shares only minimal homology. A combination of the presumed high frequency of interspecific pairing of the F_1 hybrids with the fertility of the sesquidiploids or their back-cross derivatives may elevate the actual rate of interspecific gene transfer.

In meiosis of autotriploids, the total length of the unpaired segments

adds up to a complete unpaired genome, because only two chromosomes may pair at any single point. The unpaired genome is available for pairing without competition if alien chromosomes are present in the nucleus.

The results, reported here show a significant increase in the rate of interspecific chromosomal exchanges on the autotriploid level. Nevertheless, they should be looked upon as indicative only, since the methods employed were rather crude and could not eliminate large variability due to extraneous factors.

5. SUMMARY

When there is limited meiotic pairing in interspecific hybrids, the F_1 hybrids are sterile. In order to restore fertility, one needs to use a tetraploid recipient and produce triploid hybrids. The triploid hybrids are then backcrossed repeatedly to the recipient diploid species. By a rare *interspecific chromosomal exchange*, a desirable donor locus is incorporated into the recipient's genome, and the rest of the donor genome is eliminated. The rare occurrence of interspecific chromosomal exchanges in hybrids of distantly related species is one of the limiting factors in artificial transfer of genes between species, and hence it limits the potential gene pool at the disposal of the breeder.

The present paper describes a method that might increase the above rate of transfer in some plant species. The method consists of incorporating the alien chromosomes into an autotriploid recipient nucleus. The hypothesis that autotriploidy is more conducive to interspecific chromosome exchanges is based on the known fact that in meiosis of autotriploids only two chromosomes may pair at any single point. The total length of the unpaired segments adds up to a complete unpaired genome, which is available without competition for pairing and segmental exchange with alien chromosomes.

Experimental results showed a significant increase in the rates of interspecific chromosomal transfer at the triploid level. *Nicotiana tabacum* was the recipient species, and *N. plumbaginifolia* the donor, *Ws* (White-seedlings) being the marker gene.

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