Studies of Introgressed Loci in N. tabacum

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For some years we have been interested in the mechanism and effects of incorporating alien genetic material into the genome of *N. tabacum* (1, 2, 4). One of the possibilities we hoped to explore was that of identifying the *tabacum* chromosome into which an introduced locus was transferred. Moav (9) obtained some evidence, in *tabacum-plumbaginifolia* hybrids, that the process was preferential to some extent. In 14 presumably independent introductions of the *Ws(pbg)* locus into *tabacum*, 8 exchanges involved a particular *tabacum* chromosome and 6 others were associated with 3 additional members of the set. It seemed desirable to investigate this phenomenon further, using both related and unrelated species as donors. The present paper records some preliminary results where monosomic analysis was employed to determine the specific *tabacum* chromosome that was involved in the exchange of genetic material.

Procedure

Stocks were synthesized in which the *tabacum* chromosomes carried recessive genes in the homozygous condition, including flower color genes *wh* (white) and *co* (coral), the photoperiodic growth habit genes mm_1 mm_2 (mammoth), the genes affecting normal chlorophyll production ws_1 ws_2 (white seedling) and the genes controlling fasciation fs_1, fs_2. In addition, these carried genes in homozygous condition which had been incorporated by introgression from related and unrelated species and which covered the effects of the recessives. The former group included *N. sylvestris* and *setchellii* and the latter, *glutinosa, plumbaginifolia* and *paniculata.*

The introgressed stocks were obtained in the following way. Tetraploid *N. tabacum* plants having appropriate recessive markers were crossed with different diploid species to produce sesquidiploid hybrids. These were backcrossed to diploid *tabacum* plants homozygous for recessive genes. Individuals showing the dominant phenotype were selected for further backcrossing. When it was definitely established that the lines had reached the 24-paired condition, selected plants were self-pollinated until no further segregation was observed. The time required to obtain homozygous introgressed cultures varied in different pedigrees. Some attempts at transferring other loci have been carried along for many years but the plants with covering loci still have the constitution 24 II+1 I. It is conceivable that a pair of chromosomes has been substituted for a *tabacum* bivalent but experience has indicated that such plants are recognizable morphologically. In cultures where the introgressed locus has become incorporated in the chromosome known to carry the corresponding recessive, it is also possible that the chromosomal segment has replaced the recessive locus. Since the primary objective was to identify the recipient chromosome, however, this is of little significance. Nevertheless, in investigations of the white-seedling character, it has been shown that plants maintain the homozygous condition for ws_1 ws_2 for many years. The *Ws* chromosomal segment from *N. plumbaginifolia* was eliminated in certain cell lineages resulting in green-white mottling in each generation.

Plants monosomic for the chromosome known to bear the recessive loci were pollinated by members of the appropriate stock, e.g. haplo-C x *Wh stc* (non-white from *N. setchellii*). Monosomic plants in the progeny were crossed with homozygous recessives and these populations were classified as to association of the monosomic condition with the recessive phenotype. If the normal flower-color factor in the above cross had entered the C-chromosome the resulting monosomic individuals could not have had a normal gene and they would all be white-flowered; the disomics would all have *Wh stc* and would be colored. If the gene had become associated with a chromosome other than the C-, four types would result, viz. haplo-C white, haplo-C colored, diplo-C white and diplo-C colored.

Results

Table 1 summarizes the results to date and, while they are admittedly of a preliminary nature, some significant points are evident. In population 1 it is clear that *Wh stc* has become associated with some chromosome other than the C. Since *stc* is a member of the *tomentosa* assemblage its genome should include a chromosome having a high degree of homology with C (a member of the *tomentosa* genome of *tabacum*, 3). Thus, it might have been anticipated that an exchange involving this chromosome had occurred when introgression of *Wh* from *stc* into

tabacum took place. The reason for inequality (9:16) in the disomic class is obscure as there is no evidence for differential viability in white- and carmine-flowered (non-white) classes. The discrepancy may be the result of random fluctuation.

An even greater difference was met with in the disomic classes of population 2, a result for which no explanation is readily available. The numbers recorded in the monosomic columns, of course, reflect the ovular transmission of the monosomic type under consideration but disomic plants of the alternative flower-color types were expected to occur in equal numbers. In this test again, presence of four classes shows that the F-chromosome was not the recipient of the introgressed segment.

The results in population 3 show pretty definitely that the F-chromosome took part in the transfer of *Mm* from *glutinosa* to the *tabacum* genome. Although three mm_1 mm_2 plants did not flower soon enough to be classified, the relatively large number of haplo-F mm_1 nm_2 plants and the complete absence of monosomics among the *Mm*-normals provide strong evidence for the above conclusion. This result was somewhat unexpected considering the lack of homology between *glutinosa* and *tabacum* chromosomes (8), the expectation being for purely random exchange between members of these chromosome sets. The fact that in some earlier experiments a specific chromosome (H) was replaced by, or exchanged material with, the *Nc* chromosome of the same species (6, 7) may suggest the presence of small homologous regions, especially since in each of these instances it has been a "*tomentosa*" chromosome of *tabacum* that has participated in the exchange.

The situation in the haplo-P *Fs* cultures is according to expectation based on species relationships. The lack of haplo-P fs_1 fs_2 plants in population 6 merely reflects the low transmission of this monosomic type. The absence of recessives among the disomic progeny in population 7 as compared with the large number in 6 indicates that the related species, *sylvestris*, has exchanged a segment with the P-chromosome of *tabacum* (a member of the "*sylvestris*" genome). On the other hand, the unrelated species, *glutinosa*, has contributed a segment to some chromosome other than P, presumably with one of the "*tomentosa*" members. Haplo-D would be the logical one to test first, since there is some evidence that one of the duplicate loci, fs_1, is located on this chromosome.

Table 1. Associations of Introgressed Loci with Specific Chromosomes of *N. tobacum*

	2n-1		2n		
Population	rec.	dom.	rec.	dom.	Σ
1. C-Wh stc*	6	7	9	16	38
2. F-Co glt	3	1	5	31	40
3. F-Mm glt	15+(3)**	0	**	29	47
4. G-Ws pbg	some***	0	***	38	38+
5. G-Ws pnc	0	2	0	43	45
6. P-Fs glt	0	2	21	26	49
7. P-Fs slv	2	0	0	36	38
8. T-Ws pnc	5****	2	****	34	41

* *C = monosomic type; Wh = non-white; stc = setchellii. A single exchange occurred between stc and tabacum chromosomes.*

** *Three mammoth plants did not flower in time for haplo-F classification.*

*** *Seeds were sown in excess and relative numbers of white and green seedlings could not be counted.*

**** *White seedlings died at the cotyledon stage so their chromosomal constitutions could not be determined.*

The white seedling series requires somewhat more detailed discussion since, as the double recessive is a seedling lethal, it is necessary to employ heterozygotes for hybridization. It is controlled by duplicate factors (5) but in Red Russian tobacco (in which the monosomic types were isolated) the genotype is G (ws_1/ws_1) T (Ws_2 Ws_2).

In population 5 no white seedlings appeared on the seed germinators. This result could be obtained only if the exchange between *paniculata* and *tabacum* chromosomes involved the T-member of the latter genome. If the G- or some other chromosome of the complement had been the recipient, a small percentage of white seedlings should have emerged among the monosomic offspring. Haplo-G is one of the lowest in ovular transmission so the lack of white seedlings might be attributed to the absence of haplo-G plants in this limited sample. White seedlings would not be expected among the disomics on any basis. Consequently, a large excess of seeds were germinated and, again, there were no albino seedlings among the progeny.

The results in the other white seedling cultures (Populations 4, 8) were not as conclusive as in the above. In the *plumbaginifolia* transfer, some albinos appeared. Germination was poor in the lot where seeds were placed individually on the filter paper germinator, one albino and 21 green seedlings emerging. An excess of seeds were sown in a second trial and a few more white seedlings were obtained. With this method an accurate count of white and green is impossible. No albinos would be expected among the disomics. If we accept 5 per cent as the frequency of white seedlings, a higher than usual transmission of haplo-G would be indicated since only one-fourth of the monosomic progeny are expected to be white. The facts that no haplo-G green plants were obtained and that an even higher transmission of the monosomic would be implied if some other chromosome of the set had exchanged material, support the view that the G-chromosome was indeed the recipient of the *Ws(pbg)* locus.

The same introgressed line of *Ws(pnc)* was used in producing cultures 5 and 8 so the results of the latter are merely confirmatory. Assuming that the T-chromosome was involved, it would again seem that too many albinos were present in the light of the usual low values for transmission of haplo-T and the fact that white plants should not occur among the disomics. If any chromosome other than the T- had received the *Ws(pnc)* segment a considerable proportion of both monosomic and disomic plants should have been white. All in all, the results are consistent with the hypothesis that the locus was introduced into the T- chromosome.

Discussion

It would be too time and space consuming to subject the available introgression lines to complete monosomic analysis. It has been possible, however, to derive some information on preferential chromosome exchange between other species and *tabacum* using selected monosomic types.

Unfortunately, only two species, *sylvestris* and *setchellii*, which can be considered ancestral to *tabacum* were included in the present study. In the former, exchange had occurred between a *sylvestris* chromosome

the *tabacum* unit carrying the corresponding recessive gene. In the *setchellii* instance, exchange had involved a chromosome other than the C-. It would be of interest to ascertain whether some other member of the *tomentosa* genome had been the recipient or whether the exchange was of a non-homologous nature.

While *N. glutinosa* has been included in the *tomentosa* group of species, it is much less closely related to the other four species than they are to each other. There is very little chromosome homology between *glutinosa* and present-day *tabacum* but there is some pairing in *glutinosa-tomentosiformis* hybrids. In the present tests where *glutinosa* was the donor species the results were conflicting. The *Mm* locus has been transferred to the $F(mm_1)$ chromosome but the *Co* region has become associated with some chromosome other than F(*co*).

The species, *plumbaginifolia* and *paniculata* are even less closely related to *tabacum* and the results here are too limited to indicate an association with either *sylvestris* or *tomentosa* genomes. The exchanges in these instances may have been at random although Moav (9) has obtained some evidence for non-random exchange in *plumbaginifolia-tabacum* hybrids.

Summary

1. Eight introgressed lines where loci from five related and unrelated species were introduced into *tabacum* chromosomes were investigated. The primary purpose was to determine whether chromosome exchange regularly involved the *tabacum* chromosome known to bear the recessive locus corresponding to the one introduced, using selected monosomic types.

2. Introgressed loci from related species, *sylvestris* and *setchellii* were identified with the homologous chromosome in the former and with a non-homologue in the latter.

3. Results were inconsistent where *N. glutinosa* was the donor species. In one instance the exchange involved the *tomentosa* chromosome bearing the corresponding recessive. The haplo-F(*co*) population gave evidence that some chromosome other than the F- had received the *Co* segment. This might have been expected in view of the small amount of homology between *glutinosa* and *tabacum* chromosomes.

4. The situation, where even less closely related species were employed, is considered briefly.

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