INHERITANCE IN NICOTIANA TABACUM: XXIII. DUPLICATE FACTORS FOR CHLOROPHYLL PRODUCTION

R. E. CLAUSEN AND D. R. CAMERON
University of California, Berkeley, California

Received April 7, 1949

WE HAVE previously reported (CLAUSEN and CAMERON 1944) studies of a chlorophyll deficient type, white-seedling, produced by GOODSPEED (1930) by X-ray treatment, which is inherited as a simple recessive in Purpurea, our standard variety of *Nicotiana tabacum*. In the 1946 planting season, however, it was observed that a varietal cross of Purpurea×Chinchao gave F_2 segregation in a ratio approximating 15 green:1 white-seedling. Fortunately this hybrid had been retained so that it was still available after segregation for white-seedlings had been observed in its offspring and we also had at the same time a standard Purpurea plant heterozygous for white-seedlings. The immediate progeny from intercrosses between the two, segregated for white-seedlings in a 7:1 ratio (table 2, Test cross), as was to be expected if the two types were identical genetically. These results establish a duplicate factor system for inheritance of green vs. white-seedlings.

LOCATION OF THE FACTORS, Ws_1 AND Ws_2

In a previous report (CLAUSEN and CAMERON 1944) we have outlined the procedure employed in using monosomics for determining chromosome locations of factors. The application of this scheme to the two factors concerned in white-seedling inheritance may be outlined as follows. The monosomics are of the standard variety, Purpurea, which has the gametic formula, ws_1Ws_2, whereas Chinchao is Ws_1ws_2. Application of the monosomic method of determination first involves crossing the Purpurea monosomics individually with Chinchao, then testing progenies of the selfed F_1 monosomic types separately for white-seedling segregation. Let X and Y represent the two chromosomes concerned. Then F_1 haplo-X will have the constitution

$$\frac{X_0}{X - Ws_1}\ \frac{Y - Ws_2}{Y - ws_2},$$

and F_1 haplo-Y will be

$$\frac{X - ws_1}{X - Ws_1}\ \frac{Y_0}{Y - ws_2},$$

X_0 and Y_0 representing the missing X and Y chromosomes respectively. The normal hybrid selfed will segregate, 15 green:1 white-seedling, whereas F_1 haplo-X will not segregate at all for white-seedlings, since offspring must have at least one X-chromosome, and F_1 haplo-Y will segregate, 3 green:1 white-

TABLE 1

White-seedling segregation in monosomic hybrids of Nicotiana tabacum var. Purpurea×var. Chinchao.

TYPE & GARDEN NO.	NO. OF SEEDLINGS	WHITE SEEDLINGS	PER CENT OF WHITE SEEDLINGS
haplo-A 48381p20	94	7	7.4
B 48382p12	93	8	8.6
C 48383p21	99	5	5.0
D 48384p5	87	4	4.6
E 48385p16	100	8	8.0
F 48386p11	84	7	8.3
diplo-G 48387p6	97	7	7.2
haplo-G 48387p30	80	0	0.0
	86	0	0.0
H 48388p16	95	8	8.4
diplo-I 48389p1	92	4	4.3
haplo-I 48389p26	75	0	0.0
	31	0	0.0
haplo-I 48389p32	87	1	1.1
	87	4	4.6
haplo-I 48389p40	85	6	7.1
J 48390p43	85	6	7.1
K 48391p3	98	4	4.1
L	—	—	—
haplo-M 48393p6	91	3	3.3
N 48394p14	97	5	5.2
O 48395p16	96	8	8.3
P 46372p4	99	10	10.1
P 46372p10	97	3	3.1
Q 48397p20	99	5	5.1
R 48398p11	98	7	7.1
S 48399p16	91	3	3.3
diplo-T 48400p46	98	9	9.2
haplo-T 48400p33	90	24	26.7
U 48401p11	79	7	8.9
V 48402p16	99	3	3.0
W 48403p26	93	7	7.5
Z 48404p5	93	7	7.5

seedling, since it is heterozygous for only one of the two loci. Obviously monosomics of chromosomes other than X and Y will segregate according to the duplicate factor scheme, 15 green:1 white-seedling and normal sibs of the monosomic types should segregate in the same ratio.

Results of segregation in the monosomic types are contained in table 1. The selected monosomic types were selfed and the seedling segregation ratios were determined by germinating 100 seeds in a water germinator, except in the case of resowing of 48389p26, for which only 45 seeds were available for the retest. All the monosomics except G and T segregate in satisfactory agreement with the 15:1 ratio. Of the two monosomics which behave exceptionally, haplo-G failed to segregate for white-seedlings and haplo-T gave a ratio of

3:1 instead of 15:1. The normal sibs in each case segregated in the 15:1 ratio, as was to be expected. The results conclusively demonstrate that the Ws_1 locus is located in the G chromosome, and Ws_2 in T. The latter result confirms our previous observation (CLAUSEN and CAMERON 1944), since, as a matter of fact, haplo-T had been isolated deliberately from an asynaptic type by using white-seedling as indicator of its presence in the cultures. These results unfortunately are somewhat marred by failure of the original haplo-I selection to segregate for white-seedlings. Two other haplo-I selections, however, were available, both of which segregated in the expected ratio. In conducting these experiments the original scoring of plants into monosomic and normal categories was based upon morphological features. However, each

TABLE 2

Segregation for white seedlings in various lots of comparable composition.

LOT		GREEN SEEDLINGS	WHITE SEEDLINGS	PER CENT WHITE SEEDLINGS
Varietal crosses	observed	626	44	6.6
	expected 15:1	628	42	6.25
Monosomic tests	observed	2090	136	6.1
	expected 15:1	2087	139	6.25
Diploid sibs	observed	267	20	7.0
	expected 15:1	269	18	6.25
Test cross	observed	87	14	13.9
$Ws_1ws_1Ws_2ws_2 \times ws_1ws_1Ws_2ws_2$	expected 7:1	88	13	12.5
Haplo-T segregation	observed	66	24	26.7
	expected 3:1	68	22	25.0

such selection was later verified by cytological examination. It is usually not possible to await the results of this verification for seed collection, consequently the practice has been to make the necessary pollinations immediately and to collect seed from all selected plants. In the case of haplo-I, the cytological determinations indicate that haplo-I 48389p26 had the IM association, 17(2)+6+4+2(1); i.e., there were 17 bivalents, a ring of six, a ring of four and 2 univalents. This plant therefore was a double monosomic, apparently by rare chance, haplo-G haplo-I. Haplo-I 48389p40 was 18(2)+6+4+1, an unmodified haplo-I, whereas haplo-I 48389p32, although also 18(2)+6+4+1, had some undefined complication. The normal configuration of this hybrid is 19(2)+6+4, due to structural difference between the two parental varieties, Purpurea and Chinchao; but fortunately the chromosomes carrying Ws_1 and Ws_2 are not involved in these complications.

We have collected in table 2 the totals of segregating populations and have entered also the expected segregation values. In this table the monosomic lot

contains the totals for white-seedlings in all but haplo-G and haplo-T. The diploid sibs lot records the totals of segregation observed in normal sibs of haplo G, I, and T, individually recorded in table 1. The haplo-T segregation ratio is also included, and shown, like the rest, to be in very satisfactory agreement with expectation.

THE CONSTITUTION OF *N. tabacum* VARIETIES

The demonstration that duplicate factors govern white-seedling segregation in the varieties Chinchao and Purpurea has aroused our curiosity as to the constitution of other varieties of *N. tabacum*. Obviously varieties may be of three categories, viz., duplex Ws_1Ws_2, simplex Ws_1ws_2 as in Chinchao, or simplex ws_1Ws_2 as in Purpurea. To determine the constitution of other varieties, parallel crosses were made of these varieties with Chinchao and Purpurea as testers; the F_2 results, as shown in table 3, are then diagnostic as to the constitution of the opposed varieties. The total segregation values for the crosses which produced white-seedlings are entered in table 2 as the varietal crosses lot. They are obviously in very satisfactory agreement with 15:1 expectations.

TABLE 3

Diagnostic behavior of classes of varieties of N. tabacum, when crossed with the two tester varieties, Chinchao and Purpurea.

TESTER VARIETIES	CONSTITUTION OF UNKNOWN		
	Ws_1Ws_2	Ws_1ws_2	ws_1Ws_2
Purpurea ws_1Ws_2	F_1 $Ws_1ws_1Ws_2Ws_2$ F_2 1 green:0 white	F_1 $Ws_1ws_1Ws_2ws_2$ F_2 15 green:1 white	F_1 $ws_1ws_1Ws_2Ws_2$ F_2 1 green:0 white
Chinchao Ws_1ws_2	F_1 $Ws_1Ws_1Ws_2ws_2$ F_2 1 green:0 white	F_1 $Ws_1Ws_1ws_2ws_2$ F_2 1 green:0 white	F_1 $Ws_1ws_1Ws_2ws_2$ F_2 15 green:1 white

As a result of these tests 25 varieties proved to be Ws_1Ws_2; 1 variety, Chinchao was Ws_1ws_2; and 8 varieties, inclusive of Purpurea, were found to be ws_1Ws_2, as shown in the following lists:

Ws_1Ws_2: Acomayo Red, Alameda Red, Ambalema, Apolo Pink, Apolo Red, Bergerac Catacorolla, Cachicadan, Canchaque, Cerro Alegre, Chulque Pink, Chulque White, Coquimbo, Coripata, Coroica, Cuba White, Huadquina, Huanuco, Marcapata, Maryland Mammoth, Mirador, Papago, Samsoun, Serrate, Trelease Turkish, Trujillo.

Ws_1ws_2: Chinchao

ws_1Ws_2: Belge, Ceniza, Consolation, Glutinosa-Resistant Kentucky Burley No. 1, GR Kentucky Burley No. 2, Holmes GR Samsoun, Purpurea, Station Standup Burley.

Thus most of the varieties in this relatively small sample, still possess the unmodified duplicate condition.

To what extent the eight varieties listed as ws_1Ws_2 are of independent origin

is unknown. However, three of them were burley varieties, presumably of common derivation. One of the other varieties was Holmes Glutinosa-Resistant Samsoun. Since the original Samsoun proved to be Ws_1Ws_2 and Holmes GR Samsoun was established by crossing Samsoun with an amphidiploid *glutinosa-tabacum* type derived from Purpurea, Holmes GR Samsoun must owe its ws_1 Ws_2 constitution to transfer of the ws_1 gene from Purpurea. We have no means of judging how the ws_1Ws_2 constitution may have been established in the other varieties of this class; whether by inheritance or by independent mutation; but probably the disparity in frequency of the two simplex types is not so great as that indicated by the raw data.

DISCUSSION

If as seems well established, *N. tabacum* originated as an amphidiploid hybrid, then as a raw amphidiploid it must have contained an immense number of duplications, particularly of factors concerned with vital functions, as in this instance. In the amphidiploid, therefore, it should be possible for one member of each set of duplicate factors to mutate without impairing the operation of the system. In consequence, mutation pressure in the absence of counter selection might in time lead to loss of one or the other factor so that ultimately the species would become simplex as respects its duplicate factors; i.e., reduced to the constitution, A_1a_2, speaking in general terms. In such circumstances, FISHER (1935) has shown that under panmictic conditions, the dominant gene with the higher mutation rate would disappear and the population would become simplex for the remaining dominant.

In the autogamous species *N. tabacum*, however, this process should lead to establishment of two different simplex types approximately in proportion to their respective mutation rates. Thus if mutation rates u_1 and u_2 be assumed for factors A_1 and A_2, respectively, the relative gene frequencies after n generations, in the entire absence of selection, would be as follows:

$$A_1 : a_1 = e^{-u_1 n} : 1 - e^{-u_1 n}$$

$$A_2 : a_2 = e^{-u_2 n} : 1 - e^{-u_2 n}.$$

If we ignore heterozygous conditions, which would be exceedingly rare under self fertilization, and assume that mutations at the two loci are independent and equal in rate, and allow for the elimination of double homozygous recessives, the approximate relative proportions of surviving genotypes would be:

$$\text{Duplex} \quad A_1A_2 = e^{-2un}$$

$$\text{Simplex} \quad A_1a_2 + a_1A_2 = 2e^{-un}(1 - e^{-un}).$$

Table 4 records the proportions of simplex lines to be expected in each set of duplicate factors, assuming that mutation frequency has been equal at each of the two loci. Conversely, these values may be taken to represent the relative proportions of sets of duplicate factors which have been reduced to the simplex condition under the stipulations indicated. We gratefully acknowledge the assistance of our colleague, DR. EVERETT R. DEMPSTER, in this portion of our studies.

The surprising feature of these results is the slowness with which reduction to the simplex condition will take place, as a consequence of mutation pressure alone. Mutation rates of the order of one in a million would only lead to establishment of simplex conditions in two percent of the lines after 10,000 generations. Since *N. tabacum* is an amphidiploid species, presumably established under cultivation, it is improbable that it has existed any longer than that. Moreover we have no reason to suspect that it has a mutation rate higher than

TABLE 4

Proportion of simplex lines to be expected in the surviving population under stipulated assumptions as to mutation rate and number of generations.

MUTATION RATE	PROPORTION OF SIMPLEX LINES IN n GENERATIONS		
	$n=2000$	$n=5000$	$n=10{,}000$
$u=10^{-4}$	.307	.565	.775
$u=10^{-5}$	.039	.093	.174
$u=10^{-6}$	.004	.010	.020

that characteristic of other organisms. We have, however, some cogent evidence, derived particularly from monosomic studies, which indicates that numerous sheltered lethals, to use FISHER'S term, do exist in the species, probably in high enough frequency to be present in every chromosome. As a matter of fact, even if only one percent of originally duplicate loci scattered at random throughout the genetic system became reduced to the simplex condition, then possibly enough sheltered lethals would exist to account for the lethal effect of complete loss of any large segment of the germinal material.

Our experience with the F chromosome may be pertinent to this discussion. If we remove the non-satellited arm of the F chromosome by fragmentation, and replace it with material derived from another chromosome, the result is a viable type. If the other arm of the chromosome is removed, results seem to differ depending upon the size of the deletion. If it is large, removing most of the arm, a lethal effect is obtained; if, however, it is not quite so large, a non-lethal type is produced. Here the results seem to indicate the presence of a single sheltering factor lying close to the centromere in the satellited arm of the chromosome. Deletion of segments which do not contain this factor, do not lead to lethal effects, although they may lead to other, less-pronounced reductions in viability. Thus the process of diploidization, assumed to occur in amphidiploids, operates slowly and probably does not lead to actual diploidization to the extent that substantially all sets of duplicate genes are reduced to the simplex condition, but may proceed far enough even with a low mutation rate and a moderate number of generations to assure that each effective segment will contain a sheltering factor, which is all that is necessary to account for the genetic phenomena characteristic of established amphidiploids. This probably accounts for our failure to uncover other types of chlorophyll defect, in these crosses between normal green varieties. It also probably accounts for our ex-

perience that mutants sent to us as simple recessives often prove to depend upon a duplicate factor basis when tested with our varieties.

Presence of duplicate factors for chlorophyll production has recently been observed by MATSUMURA and KONDO (1942) in crosses between races of the tetraploid species, *Aegilops triuncialis*. PHILP (1935) in some earlier observations in effect demonstrated existence of duplicate loci for albinism in hexaploid oats *Avena sativa*. Recently AUSEMUS, HARRINGTON, REITZ, and WORZELLA (1946) have summarized observations of the occurrence of sets of duplicate factors in tetraploid and hexaploid wheat. None of these studies, however, reveals any information as to the frequency of the genes in question.

SUMMARY

1. In *N. tabacum*, crosses between the normal varieties Chinchao and Purpurea segregate in a 15:1 ratio for green vs. white-seedlings in F_2, thus demonstrating existence of duplicate factors for chlorophyll production.

2. The white-seedling type for which this cross segregates is shown to be identical with or allelic to the white-seedling type established by GOODSPEED (1930) by X-ray treatment of Purpurea.

3. Monosomic tests demonstrate that one of the loci is located in the G and the other in the T chromosome.

4. A study of the constitution of a group of varieties revealed that 25 were Ws_1Ws_2, 1 Ws_1ws_2, and 8 ws_1Ws_2.

5. Attention is called to FISHER's (1935) theoretical treatment of the problem of sheltering of lethals in tetraploids, which leads to the conclusion that mutation pressure alone is relatively ineffective in reducing duplicate factor sets to a simplex condition. This conclusion appears to be substantially in agreement with conditions in amphidiploid *N. tabacum*, as far as present scanty evidence is concerned.

LITERATURE CITED

AUSEMUS, E. R., J. B. HARRINGTON, L. P. REITZ, and W. W. WORZELLA, 1946 A summary of genetic studies in hexaploid and tetraploid wheats. J. Amer. Soc. Agron. **38**: 1082–1099.

CLAUSEN, R. E., and D. R. CAMERON, 1944 Inheritance in *Nicotiana tabacum*. XVIII. Monosomic analysis. Genetics **29**: 447–477.

FISHER, R. A., 1935 The sheltering of lethals. Amer. Nat. **69**: 446–455.

GOODSPEED, T. H., 1930 Inheritance in *Nicotiana tabacum*. IX. Mutations following treatment with X-rays and radium. Univ. Calif. Publ. Bot. **11**: 285–298.

MATSUMURA, S., and N. KONDO, 1942 Varietätsbastarde bei *Aegilops triuncialis* L. Bot. Mag. Tokyo **56**: 225–234. (Japanese with German résumé.)

PHILP, JAMES, 1935 Aberrant albinism in polyploid oats. J. Genet. **30**: 267–302.