Radiation-induced Interspecific Transfer of Ws(pb) Gene from *Nicotiana plumbaginifolia* to *N. tabacum*.

I. Some observations on the irradiated hybrid plants.\(^1,2\)

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**Synopsis.** Soaked seeds of a *Nicotiana* hybrid plant, of which chromosome constitution was 24 pairs of *tabacum* chromosomes plus one *plumbaginifolia* chromosome, were irradiated by 5,000 R of X-ray. A-dominant gene Ws(pb) translocated from *plumbaginifolia* chromosome to *tabacum* chromosome. Some discussions are made about chimaeric constitution of X1 plants. The rate of the translocation was about 5%.

**Introduction**

Many works have been done to introduce useful alien genes, apart from other useless genes, to some cultivated speies. The difficulty in this breeding program lies in low rate of interspecific or -generic gene exchanges due to low frequency of interspecific or -generic chromosome pairing during meiosis.

Irradiating intergeneric hybrids of wheat, some workers have succeeded in transferring alien chromosome segments which carry useful gene (or genes) to wheat chromosomes (Osborn and Elliott 1955, Sears 1956, Elliott 1957, Knott 1961, Driscoll and Jensen 1963).

Though several valuable varieties of tobacco have been bred by means of introducing useful genes from wild species to cultivated one (Clayton 1958, Oka 1961), none of them have been bred by irradiating hybrids or hybrid derives.

In the present series of experiments, it was intended to transfer alien chromosome segment from wild to cultivated species of *Nicotiana* by means of radiation induced chromosome breakage and reunion. In this paper, some results of induced interspecific gene transfer by irradiation of seeds of hybrid derivatives are described.

**Material and Methods**

A recessive allele *ws* of *Nicotiana tabacum* (n=24) conditions an inability to produce chlorophyll (Claussen and Cameron 1950). The dominant gene Ws(pb) of *N. plumbaginifolia* (n=10) covers the effect of *ws*. Introduced by hybridization into a *tabacum* nucleus, the *plumbaginifolia* chromosomes become somatically unstable and are often lost during somatic cell divisions, so that the hybrid plants bearing recessive homozygous state on the Ws locus in their *tabacum* genomes are usually mottled (Fig. 1)

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1) Contribution from Plant Breeding Laboratory, Faculty of Agriculture, University of Tokyo, No. 52.  
2) A part of Doctor Thesis Submitted to University of Tokyo in 1964.  
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(Moav 1957). But when two plumbaginifolia chromosomes carrying $Ws(pbg)$ gene are included in the hybrids, the plants show self green color, because the probability that both $Ws(pbg)$ genes are lost from a nucleus is very low. It is also said that when $Ws(pbg)$ gene is introgressed into tabacum chromosome the plant shows usually self green color (Moav 1957).

About 3,000 seeds were obtained by self pollinating a mottled tabacum-plumbaginifolia hybrid derivative plant of which chromosome constitution was 24 pairs of tabacum chromosomes plus 1 plumbaginifolia chromosome and meiotic configuration was $24^{n} + 1^{1}$. Soaked into water at 20°C for 24 hours, they were irradiated by 5,000 R of X-ray at a dose rate 500 R/min. X-irradiation was operated at 190 kVp and 25 mA using a 1 mm Al-filter. After germination, some of the seedlings were transplanted to pots and grown in a green house.

Cytological observations on pollen mother cells of $X_1$ plants were carried out with acetocarmine smear method.

### Results

About 350 green seedlings were segregated from irradiated seeds, and self green seedlings were rejected out of the observation as they were thought to have two plumbaginifolia chromosomes and it was difficult to distinguish in the seedling stage whether the self green leaves were due to the interspecific transfer of $Ws(pbg)$ gene or due to the duplication of plumbaginifolia chromosomes.

On some mottled $X_1$ plants, self green leaves and leaves with self green sectors were observed. Those leaves appeared on the fifth to the eight leaves at first and all the lower leaves to them were mottled. They are shown photographically in Fig. 2 and schematically in Figs. 3 and 4. It will be seen, from the Figs. 3 and 4, that the self green and sectored leaves developed almost to the same directions of the stems.

Figs. 3 and 4 show different patterns of self green “chimaera” of $X_1$ plants. Both self green and mottled parts were observed still in upper cycle of phyllotaxis of the $X_1$ plant shown in
Figure 3. Scheme showing a pattern of self green chimaera. I, II, III and IV designate cycles of phyllotaxis and 8,9,10, etc. show position of leaves. Dotted parts correspond to mottled parts. Both mottled and self green parts are observed in upper cycles of phyllotaxis.

Fig. 3, while upper cycles of phyllotaxis of the X₁ plant shown in Fig. 4 consisted of only self green leaves.

These relationships are graphically shown in Fig. 5. It is seen that in 6 of 8 plants, upper cycles of phyllotaxis consist of only self green leaves and in the other 2, mottled and sectored leaves were observed still in upper cycles of phyllotaxis.

Some of the X₁ plants branched after differentiation of the 5th or 6th leaves, and in some of those branched plants self green leaves appeared on one of the branches, while only mottled leaves developed from another branch. One of such plants is photographically shown in Fig. 6.

Meiotic configurations were observed in pollen mother cells of some self

Figure 4. Scheme showing a pattern of self green chimaera. All the upper leaves of this plant show self green color (cf. Fig. 3).

Figure 5. Changes of percentage of area of self green parts in a cycle of phyllotaxis.
green sectored X₁ plants. Three to seven flower buds were taken from each plant. The result is presented in Table 1. Plants designated as 50-1, 50-2, 50-4, 50-7, 5 C-1, 5 C-6 and 5 C-8 are those of which all the upper leaves showed self green color. Except 50-2, no univalent chromosome was observed in their pollen mother cells. In these plants, uniform configurations were observed throughout all the flower buds of a plant. On the contrary, two kinds of configurations were observed in the plant 50-6 which developed self green, mottled and sectored leaves still in upper cycles of phyllotaxis. $24_H + 1_I$ configuration was observed in pollen mother cells developed axillary from mottled leaves. The plants 5 C-9 and 5 C-2 branched after differentiation of the 5th leaf, and one of the branches developed self green leaves. $24_H$ and $23_H + 1_I$ configurations were observed in pollen mother cells of self green branches, while $24_H + 1_I$ configurations were observed in those of mottled branch.

The chimaeric X₁ plants were self pollinated and segregation for leaf color in X₂ generation was observed on seedlings which were germinated on wet filter paper in petri dishes. The results are summarized in Table 2. It should be noted that the percentage of green seedlings are considerably different not only

### Table 1. Meiotic configurations in PMC's of some chimaeric X₁ plants

<table>
<thead>
<tr>
<th>Plant Designation</th>
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<th>Plant Designation</th>
<th>Configuration</th>
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<tbody>
<tr>
<td>50-1</td>
<td>24₁</td>
<td>50-6*</td>
<td>24₁ + 1₁</td>
</tr>
<tr>
<td>50-2</td>
<td>24₁ + 1₁</td>
<td></td>
<td></td>
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<tr>
<td>50-4</td>
<td>24₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-7</td>
<td>22₁ + 1₁₁₁</td>
<td>5 C-9** (Self Green)</td>
<td>23₁ + 1₁₁₁</td>
</tr>
<tr>
<td>5 C-1</td>
<td>24₁</td>
<td>5 C-9** (Mottled)</td>
<td>24₁ + 1₁</td>
</tr>
<tr>
<td>5 C-6</td>
<td>23₁ + 1₁₁₁</td>
<td>5 C-2** (Self Green)</td>
<td>24₁</td>
</tr>
<tr>
<td>5 C-8</td>
<td>24₁</td>
<td>5 C-2** (Mottled)</td>
<td>25₁ + 1₁</td>
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* Sectored and mottled leaves were observed in upper cycles of phyllotaxis. ** Stems branched and one of two branches was self green.

### Table 2. Segregation for leaf color in progenies some X₁ chimaera plants

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<tr>
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<tbody>
<tr>
<td>50-1</td>
<td>22 34</td>
<td>43 66</td>
<td>-</td>
<td>65</td>
</tr>
<tr>
<td>50-6</td>
<td>70 64</td>
<td>40 36</td>
<td>-</td>
<td>110</td>
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<td>52 68</td>
<td>14 18</td>
<td>10 13</td>
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<tr>
<td>45 59</td>
<td>31 41</td>
<td>-</td>
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<td>32 53</td>
<td>28 47</td>
<td>-</td>
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<td>52 50</td>
<td>52 50</td>
<td>-</td>
<td>104</td>
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</tr>
<tr>
<td>50-8</td>
<td>77 55</td>
<td>63 45</td>
<td>-</td>
<td>140</td>
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<tr>
<td>57 45</td>
<td>70 55</td>
<td>-</td>
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<td>47 51</td>
<td>46 49</td>
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<td>93</td>
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<tr>
<td>50-9</td>
<td>48 75</td>
<td>16 25</td>
<td>-</td>
<td>64</td>
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<tr>
<td>50-10</td>
<td>21 29</td>
<td>52 71</td>
<td>-</td>
<td>73</td>
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<tr>
<td>16 38</td>
<td>25 62</td>
<td>-</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>5 C-1</td>
<td>61 45</td>
<td>74 55</td>
<td>-</td>
<td>135</td>
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<tr>
<td>33 41</td>
<td>47 59</td>
<td>-</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>5 C-9</td>
<td>109 67</td>
<td>1 1</td>
<td>2 2</td>
<td>112</td>
</tr>
<tr>
<td>(Mottled)</td>
<td>66 90</td>
<td>3 4</td>
<td>4 5</td>
<td>73</td>
</tr>
<tr>
<td>5 C-9</td>
<td>77 79</td>
<td>20 21</td>
<td>-</td>
<td>97</td>
</tr>
<tr>
<td>(Stable Green)</td>
<td>106 77</td>
<td>31 23</td>
<td>-</td>
<td>137</td>
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</table>
among plants but also among capsules within a plant. Percentage of green plants in
the progenies from self green branch of the plant 5C-9 was higher than that in the
progenies from the mottled branch. In an X₁ population of progenies originated from
one of the capsules of the plant 50-6, in which mottled and sectored leaves were
observed in upper cycles of phyllotaxis, mottled seedlings were segregated. The
mottled seedlings were also observed in two capsule populations from a mottled branch
of plant 5C-9. But all the seedlings in the other populations were self green.

It is also interesting that the percentage of green plants in progenies from 50-1, of
which pollen mother cells showed 21n meiotic configuration (Table 1), is higher than
that in self green branch of 5C-9, which showed 23n+1n configuration in its pollen
mother cells.

As a control, 202 mottled plants were grown from seeds without X-irradiation.
Neither self green sectored plants nor branched plants were found in them. Number
of mottled X₁ plants grown were 307, and 16 of them were sectored self green. Three
of 21 branched X₁ plants differentiated branches with self green leaves.

Discussion

MoaV (1957) stated that introgressed into tabacum chromosomes, Ws(pbq) usually
becomes stable and the plant shows mottling seldom. The male transmission rate of
an additional plumbaginifolia chromosome is 3% and the female one is 15%. Hence,17.5%
of progenies originated from self pollinating of an alien addition plant is
expected to be green. The transmission rate of Ws(pbq) introgressed into tabacum
chromosome is usually higher than that of Ws(pbq) on intact plumbaginifolia chromo-

No univalent chromosomes were observed in the self green sectored plants except in
50-2 (Table 1), and the percentage of green plants in the self pollinated progenies of
these self green sectored plants were higher than 17.5%, moreover no mottled seedlings
found in most of the progeny populations. All these facts suggest that translocation
of Ws(pbq) from plumbaginifolia chromosome to tabacum chromosome must have taken
place by X-irradiation of seeds.

It can be estimated that the induced translocation of Ws(pbq) took place in cells
located at shoot apices in seeds. When a mass of cells originated from the cells, in
which the interspecific translocations occurred, took great part in formation of leaves,
the self green sectors become visible on those leaves. This is thought to be why self
green sectors appeared at first on the 5th to the 8th leaves and all the lower leaves
to them were mottled (cf. Steīn and Steffensen 1959).

In some of the chimaeric plants, mottled and sectored leaves were observed still in
upper cycles of phyllotaxis (Figs. 3 and 5). Two kinds of meiotic configurations were
observed in one of those plants and mottled seedling segregated from the progenies
of one capsule of that plant. These facts indicate that an inflorescence of some X₁
tobacco plant is not genetically homogeneous, as Gaul (1959) observed in X-irradiated
barley plants.
Kuehnert (1962) reported that after irradiation of tomato seeds by thermal neutrons, the cells were damaged and two growing points were reconstructed in some shoot apices. It is supposed that the same phenomenon as in tomato took place in the branched X₁ plants, and one of the reconstructed growing points contained a cell where interspecific translocation of Ws(pbɡ) had occurred so that the leaves developed on one branch were self green.

Trivalent chromosomes observed in 5C-6 and 5C-9 indicate that reciprocal translocations took place between *tabacum* and *plumbaginifolia* chromosomes. Quadrivalent chromosomes in 50-7 may be due to reciprocal translocations between two non-homologous *tabacum* chromosomes. A univalent chromosome in 50-2 is supposed to be a centric fragment of *plumbaginifolia* chromosome because the plant 52-2 developed self green sectors and leaves after differentiation of mottled 6th leaf, but it was impossible to ascertain under microscope whether the univalent was due to a centric fragment or an intact chromosome of *plumbaginifolia*.

Sears (1956) speculated that intercalary translocation including small segments around the desirable alien gene was more hopeful than reciprocal ones, because there would be no loss of genetic material from the recipient species and very little possibility of addition of deleterious genes of the donor species. Driscoll and Jensen (1963) stated that if the lines with high transmission rate of introgressed gene were selected, the lines would include desirable translocation such as intercalary translocation.

As mentioned earlier, the percentage of green plants in the progenies originated from 50-1 was higher than that in the progenies from the self green branch of 5C-9 (Table 2). Meiotic configuration of the former was 23½ + 1m which may be due to reciprocal translocation between *tabacum* and *plumbaginifolia* chromosomes, and there was no microscopic evidence that reciprocal translocation took place in 50-1. So it is reasonable to assume that desirable translocation of Ws(pbɡ), such as intercalary, took place in 50-1.

**Summary**

A dominant gene Ws(pbɡ) of *Nicotiana plumbaginifolia* covers the effect of a recessive allele *ws* of *N. tabacum* which conditions an inability of chlorophyll production. As additional *plumbaginifolia* chromosomes are somatically unstable in *tabacum* nucleus, the hybrid plants are usually mottled.

Soaked seeds of a hybrid derivative plant of which chromosome constitution was 24 pairs of *tabacum* chromosomes (*ws ws*) plus one *plumbaginifolia* chromosome (*Ws(pbɡ)*) were irradiated by 5,000 R of X-ray. Self green sectors and leaves were observed in some mottled X₁ plants. Some of the X₁ plants were branched and self green leaves were developed on one of the branches. Meiotic configurations of these X₁ plants and segregation ratio for leaf color in X₂ generation proved that the self green sectors and leaves were due to interspecific translocations of Ws(pbɡ). It was observed that an inflorescence of some X₁ plant was genetically heterogeneous. The transmission rate of Wg(pbɡ) translocated to *tabacum* chromosome reciprocally was very low. The rate
of the induced translocation of $W_s(pbg)$ was about 5%.

Acknowledgement

The author wishes to express his sincere thanks to Prof. T. Matsuo for his valuable counsel and discussion of this work, to Prof. D. R. Cameron for his kindness to have sent seeds of Nicotiana hybrid which were used in the present experiment, and to Dr. S. Biswas for his help in preparing the manuscript.

Literature Cited


