The Origin of the Hybrid Triploid Willows Cultivated in Argentina

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In the Delta of the Parana River there are about 100,000 hectares cultivated with Salicaceae. About one third of this surface is probably cultivated with natural hybrid willows ("sauce hibrido", "sauce mestizo", etc.) (GOLFAR, 1958). The origin of these hybrids has been unknown for some time. They began to be planted about 1920 and have become extensively cultivated on account of having higher yields than Salix alba var. calva.

Recently, RAGONESE and RIAL A LBERTI (1958) have studied the morphology of these male and female hybrids. The present author (HUNZIKER, 1958) studied the chromosome behavior at meiosis of three male hybrids, which proved to be triploids (2n = 57). On the basis of the morphological and cytological data available at that time it was postulated that these hybrids had possibly originated through hybridization between the indigenous diploid willow (S. humboldtiana) and any of the cultivated tetraploids Salix babylonica and S. alba var. calva (HUNZIKER, 1958). RAGONESE y RIAL A LBERTI, 1958 a, b). The three species grow together in many places of the Delta due to the natural occurrence of S. humboldtiana and to the extensive cultivation of female trees of the two tetraploids. The fact that no male trees of S. babylonica or S. alba var. calva had been found under cultivation in the Delta suggested that S. humboldtiana was the putative male parent.

The present paper deals with the meiotic behavior of two artificial hybrids between S. babylonica and S. humboldtiana. The bearing of this additional cytogenetic information on the origin of the hybrid willows will be discussed.

Materials and Methods

The hybrids were obtained in 1958 by Ing. Agr. ARTURO RAGONESE and Mr. FLORENTINO RIAL A LBERTI as part of a project on hybridization and forest tree breeding.

Two hybrid plants were studied cytologically. These are: 160-1 = Salix babylonica 6303 X S. humboldtiana 9729. 161-4 = S. babylonica 9716 X S. humboldtiana 9729.

The origin of the parental clones is as follows:

S. babylonica, 6303 = National Center of Agricultural Research, Castelar, Prov. Buenos Aires, Argentina; 9716 = Vivero Angel Gallardo, Santa Fé, Argentina.

S. humboldtiana, 9729 = Isla Don Humberto, Paraná Delta, Entre Ríos, Argentina, col. RAGONESE-RIAL A LBERTI Nº 8 (BAB). This is a precocious flowering strain which made the cross possible.

Herbarium specimens of the hybrids and parental strains have been deposited at the Institute of Botany, National Institute of Agricultural Technology, Buenos Aires (BAB).

The cytological studies are based on acetocarmine preparations of material previously fixed in a 3:1 mixture of alcohol-acetic acid and storea in the fixing fluid at low temperature.

Before squashing the buds were soaked in 45% acetic acid for at least half an hour. After having been studied the slides were made permanent using the freezing method and mounted in euparal.

For examination of pollen fertility a 1:1 mixture of acetocarmine and glycerine was used. Data are based on counts of at least 700 pollen grains.

Cytological Results

Both hybrids were triploids as expected, having 57 chromosomes and very irregular meiosis. The data on the chromosomal associations are given in table 1. In addition to the cells listed in this table 30 cells of 160-1 and 22 of 161-4 were observed. In these not all the associations could be analyzed but about 2-8 bivalents and 1-9 bivalents, respectively, could be detected. Occasional trivalents or quadrivalents also were observed.

In 160-1 at the end of meiosis 339 sporads were observed; of these 60.2% were tetrads, 5.9% triads and 33.9% dyads (Fig. 4). Most of the fertile pollen grains were tricolporate, a few were tetracolpate.

Discussion

Most of the artificial hybrid plants (160-1, 15, 16, 161-2, 4) are morphologically very similar to the natural hybrids, especially to one of them ("sauce hibrido")1). This fact and

1) RAGONESE and RIAL A LBERTI, Personal communication.
the close agreement between the number of bivalents and univalents in the artificial and natural hybrids indicates that most or all of the latter have originated also from hybridizations between *Salix babylonica* and *S. humboldtiana*. The difference between means of univalents and bivalents in artificial and natural hybrids (pooled data, *Table I*) is not significant (t value for univalents = 1.83, 0.10 < p > 0.05; t value for bivalents = 1.13, 0.50 < p > 0.10).

The high frequency of quadrivalents observed in “híbrido” could be simply due to sampling errors on account of the small number of cells studied; in all other natural and artificial hybrids quadrivalents or trivalents were also observed, but at a lower frequency. It could also be due to the fact that *S. babylonica*, having itself multivalents and univalents at meiosis (Ferreira de Almeida, 1946) and probably being a segmental allopolyploid, could produce gametes with different chromosome rearrangements. Its hybrid progeny, therefore, could show differences in regard to translocated segments.

Morphological and cytogenetical evidence, therefore, indicates that “sauce híbrido”, and most or all other trilopid hybrids cultivated in the Delta, originated through natural hybridization between *S. babylonica* (female parent) and *S. humboldtiana* (male parent).

Artificial hybrids between *S. alba* var. calmis and *S. humboldtiana* have been produced but they are very weak and slow growing and morphologically they do not agree with any of the cultivated natural hybrids. Unfortunately it has not been possible so far to study these hybrids cytologically.

The nature of the chromosome pairing in the natural hybrids has been fully discussed elsewhere (Hunziker, 1938). With regard to the artificial hybrids we may assume, similarly, that mostly results from autosynthesis among *S. babylonica* chromosomes. Autosynthesis is not completely ruled out but several facts suggest a predominance of auto-

syndosis. First, they are intersectional hybrids and probably the genome of *S. humboldtiana* has rather little homology with any of those of *S. babylonica*. Second, Ferreira de Almeida (1946) has apparently found up to 6 quadrivalents and one trivalent in *S. babylonica*, which suggests close homology between the two genomes of this tetraploid species.

Assuming that there are no factors producing asynapsis and since no cell with 19 bivalents was observed, chromosome homology in the artificial hybrids is interpreted as segmental, whole homologous genomes not being present. Moreover, the quadrivalent observed in one of the artificial hybrids indicates the existence of translocation heterozygo-
sity due to differences in the genomes.

The origin of the different kinds of sporad and pollen grains has been discussed previously in detail for the natural hybrids (Hunziker, 1938). The same could be said of the artificial hybrids. Dyads may be formed through fusion of parallel spindles in Metaphase II or through formation of restitution nuclei at Division II.

Triad formation, on the other hand, is probably produced mostly by convergence of the two spindles to one common pole at II Division. The triad consists probably, in most cases, of one nearly triploid and two more or less reduced pollen grains. This is suggested by the unequal size of the nuclei in the triads, one of them being larger than the other two (Fig. 4).

Probably most of the fertile pollen of the artificial hybrids results from dyads. The differences in pollen fertility between plant 160-1 and 161-4 are probably only due to environmental differences. The possible influence of external as well as internal factors on the frequency of the meiotic abnormalities (spindle fusion, convergence of spindles, restitution nuclei) that lead to variable percentages of sporad and staminate pollen has already been discussed (Hunziker, 1938).

**Acknowledgements**

The author wishes to express his gratitude to Ing. A. G. Arturo Radonne and Mr. Florentino Rial Alberti who obtained the artificial hybrids and facilitated the access to this material for cytogenetic studies. Thanks are due also to Dr. R. Marie Valenzuela for reading the manuscript and to Miss Alcira Gamero for most of the statistical calculations. This work was supported by a grant from the National Institute of Agricultural Technology, Argentina (I. N. T. A.)

**Summary**

In order to obtain additional evidence concerning the origin of the triploid willows cultivated in the Delta of the

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**Table I.** Chromosome behavior at Metaphase I and pollen fertility in two artificial *Salix babylonica × S. humboldtiana* hybrids and in two natural hybrids.

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Chromosomal Associations Range and mean ± S. E.</th>
<th>Number of completely analyzed cells</th>
<th>Pollen fertility, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV</td>
<td>III</td>
<td>II</td>
</tr>
<tr>
<td>Artificial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160-1</td>
<td>0 (0-1)</td>
<td>-</td>
<td>1.8 ± 0.52</td>
</tr>
<tr>
<td>161-4</td>
<td>-</td>
<td>0 (0-1)</td>
<td>5.8 ± 0.68</td>
</tr>
<tr>
<td>Pooled data</td>
<td>(160-1, 161-4)</td>
<td>0 (0-1)</td>
<td>5.8 ± 0.68</td>
</tr>
<tr>
<td>Natural</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“mestizo” (6897)</td>
<td>-</td>
<td>0 ± 0.11</td>
<td>4.8 ± 1.00</td>
</tr>
<tr>
<td>“híbrido” (5844 y 6896)</td>
<td>0.50 ± 0.34</td>
<td>(0-1)</td>
<td>5.8 ± 1.17</td>
</tr>
<tr>
<td>Pooled data (6897, 5844, 6896)</td>
<td>1.17 ± 0.12</td>
<td>0.11 ± 0.08</td>
<td>11 (12)</td>
</tr>
</tbody>
</table>

*) The data for the natural hybrids 6897, 5894 and 6896 are from Hunziker (1938). Data within parenthesis belong to cells in which not all the chromosomal associations could be analyzed and, therefore, have not been included in the column of number of completely analyzed cells.

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* Radonne and Rial Alberti, personal communication.
Weitere Beobachtungen zum Blühen und Fruchten von Junglärchen

Von H. Wachtler, Haldensleben

(Eingegangen am 9. 4. 1963)

Einleitung


Beobachtungen


a) Calvörde, Abt. 38 7- bis 8j. Lärchen:

<table>
<thead>
<tr>
<th>Gruppe</th>
<th>Anzahl</th>
<th>Zapfen</th>
<th>arithm. Mittel der Höhentriebe (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>10–50</td>
<td>29,5, 46,2, 7,4, 71,2</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>10–50</td>
<td>29,5, 20,1, 13,9, 52,5</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>—</td>
<td>28,5, 37, -16,3, 66,8</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>—</td>
<td>26,-, 27,5, 43,-, 87,-</td>
</tr>
</tbody>
</table>

b) Seesehausen/Altmark 8- bis 9j. Lärchen:

<table>
<thead>
<tr>
<th>Gruppe</th>
<th>Anzahl</th>
<th>Zapfen</th>
<th>arithm. Mittel der Höhentriebe (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1958</td>
<td>50</td>
<td>55, 69, 83, 97</td>
</tr>
<tr>
<td>B</td>
<td>1955</td>
<td>61</td>
<td>62, 71, 81, 90</td>
</tr>
<tr>
<td>C</td>
<td>1956</td>
<td>64</td>
<td>62, 71, 81, 90</td>
</tr>
<tr>
<td>D</td>
<td>1957</td>
<td>67</td>
<td>62, 71, 81, 90</td>
</tr>
<tr>
<td>E</td>
<td>1958</td>
<td>60</td>
<td>62, 71, 81, 90</td>
</tr>
</tbody>
</table>

(Die Gruppen A–D wurden ausgeschieden, um nur Pflanzen mit einheitlicher Tendenz hinsichtlich des jährlichen Höhenzuwachses zur Mittelbildung zu verwenden.)


Außer in der Farbe der Blüten zeigten sich (nach Beobachtungen im Frühjahr 1960) Unterschiede. Der Anteil grüner Blüten lag in Seesehausen bei etwa 10%, in Calvörde bei etwa 20 bis 30%. Nach den Zapfen- und Blütenmerk-