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 Salicacaae. About one third of this surface is probably cultivated with natural hybrid willows ("sauce hibrido", "sauce mestizo", etc.) (GOLFARI, 1958). The origin of these hybrids has been unknown for some time. They began to be planted about 1920 and have become extensely cultivated on account of having higher yields than Salix alba var. calva.

Recently, RAGONESE and RIAL ALBERTI (1958) have studied the morphology of these male and female hybrids. The present author (HUNZIKER, 1958) studied the chromosomz 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 ALBERTI, 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 femnle 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 ALBERTI as part of a project on hybridization and forest tree breeding.

Two hybrid plants were studied cytologically. These are:

160-1 = Salix babylanica 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 Rios, Argentina, col. RAGONESE-RIAL ALBERTI N⁰ 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.

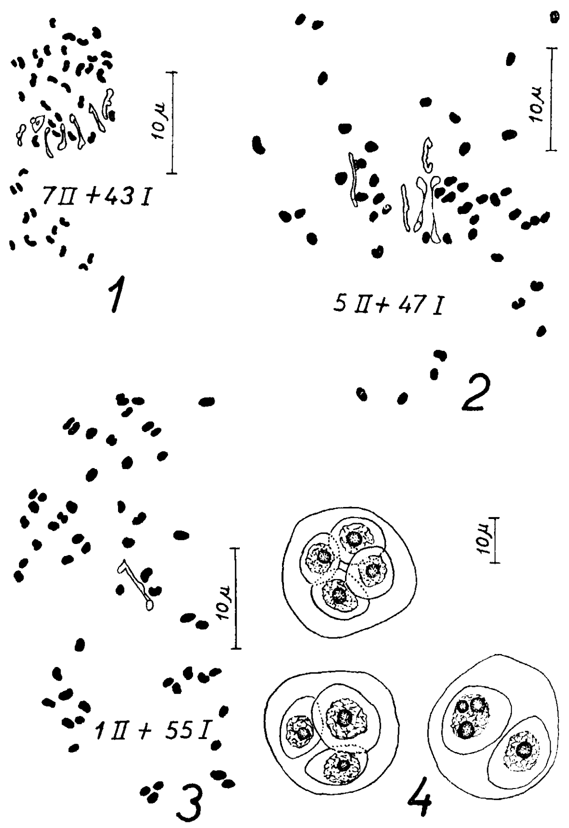
Cytological Results

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 tricolpate, 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 híbrido")[1]). This fact and



Figs. *1—4:* Meiosis in the artificial Salix babylonica × S. *humboldtiana* hybrids. — Figs. *1—2:* 161-4, Metaphase I. — Figs. *3—4:* 160-1. — *Fig.* 3: Metaphase I. — Fig. 4: Tetrad, triad and dyad at the end of meiosis. — Bivalents outlined, univalents in black.

[1]) RAGONESE and RIAL ALBERTI, Personal communication.

Table 1. — Chromosome behavior at Metaphase I and pollen fertility in two artificial *Salix babylonica* × *S. humboldtiana* hybrids and in two natural hybrids*)

	Hybrids	Chromosomal Associations Range and mean ± S. E.				Number of completely analyzed cells	Pollen fertility, per cent
		IV	III	II	I		
Artificial	160—1	(0—1) —	— —	1—8 4.00 ± 0.52	41—55 49.00 ± 1.04	22	4.2
Artificial	161—4	— —	(0—1) —	(1) 2—9 5.80 ± 0.68	39—53 45.40 ± 1.36	10	17.8
Artificial	Pooled data (160—1, 161—4)	(0—1) —	(0—1) —	1—9 4.56 ± 0.44	39—55 47.87 ± 0.87	32	4.2—17.8
Natural	„mestizo" (6897)	— —	0—1 0.17 ± 0.11	0—11 4.83 ± 1.00	35—54 46.83 ± 1.82	12	11—29
Natural	„híbrido" (5844 y 6896)	0—2 0.50 ± 0.34	(0—1) —	2—9 (12) 6 83 ± 1.17	39—45 41.33 ± 1.08	6	1.4—9
Natural	Pooled data (6897, 5844, 6896)	0—2 0.17 ± 0.12	0—1 0.11 ± 0.08	0—11 (12) 5.50 ± 0.78	35—54 45.00 ± 1.39	18	1.4—29

*) The data for the natural hybrids 6897, 5844 and 6896 are from HUNZIKER (1958). 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.

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 1*) ist 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 triploid hybrids cultivated in the Delta, originated though natural hybridization between *S. babylonica* (female parent) and *S. humboldtiana* (male parent).

Artificial hybrids between *S. alba* var. *calva* 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[2]). 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, 1958). With regard to the artificial hybrids we may assume, similarly, that mostly results from autosyndesis among *S. babylonica* chromosomes. Allosyndesis is not completely ruled out but several facts suggest a predominance of autosyndesis. 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 cuadrivalents 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 heterozygosity due to differences in the genomes.

The origin of the different kinds of sporads and pollen grains has been discussed previously in detail for the natural hybrids (HUNZIKER, 1958). 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 sporads and stainable pollen has already been discussed (HUNZIKER, 1958).

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Summary

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

[2]) RAGONESE and RIAL ALBERTI, personal communication.

Paraná River the meiotic behavior of two artificial hybrids between *Salix babylonica* and *S. humboldtiana* was studied. Meiosis was very irregular and similar in artificial and natural hybrids. As the artificial hybrids are morphologically very similar to the natural ones it is concluded that most or all of these natural hybrids result from hybridization between *S. babylonica* and *S. humboldtiana*.

Zusammenfassung

Titel der Arbeit: *Über die Entstehung der triploiden Weiden in Argentinien.*

Um weitere Hinweise über die Entstehung der triploiden Weiden zu erhalten, die im Delta des Paraná kultiviert werden, untersuchte man jetzt das meiotische Verhalten von zwei künstlichen Bastarden zwischen *Salix babylonica* und *S. humboldtiana*. Ihre Meiose war sehr unregelmäßig und derjenigen künstlicher und natürlicher Hybriden ähnlich. Da nun auch diese künstlichen Bastarde außerdem morphologisch den natürlichen sehr ähnlich sehen, wurde daraus geschlossen, daß die meisten oder auch alle diese natürlichen Hybriden aus einer Bastardierung von *S. babylonica* und *S. humboldtiana* resultieren.

Résumé

Titre de l'article: *Origine des saules hybrides triploïdes cultivés en Argentine.*

Pour obtenir de nouvelles informations sur l'origine des saules triploïdes cultivés dans le delta de la rivière Paraná, on a étudié le comportement à la méiose de deux hybrides artificiels entre *Salix babylonica* et *S. humboldtiana*. La méiose était très irrégulière et analogue chez les hybrides artificiels et chez les hybrides naturels. Les hybrides artificiels étant morphologiquement très semblables aux naturels, on en conclut que la plupart ou la totalité de ces hybrides naturels proviennent d'une hybridation entre *S. babylonica* et *S. humboldtiana*.

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Weitere Beobachtungen zum Blühen und Fruchten von Junglärchen

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Einleitung

Das Fruchten von Junglärchen kann nach Beobachtungen des Verfassers in bestimmten Fällen mit der Einwirkung von Spätfrösten im Zusammenhang stehen. Es zeigte sich nämlich, daß nur die von einem Spätfrost ernstlich betroffenen, an stärkerer Höhentriebentwicklung verhinderten Junglärchen im darauffolgenden Jahr stark fruktifizierten. Der größte Zapfenbehang war an den Bäumchen zu beobachten, deren letzter Höhentrieb im Vergleich zu dem des Vorjahres besonders gering war. Dagegen blühten und fruchteten jene Lärchen nicht, deren Höhentrieb gegenüber dem des Vorjahres eine Steigerung aufwies. Da Saatgut von anerkannten Lärchen verwendet worden war, konnte diese Frühreife nicht als Ausdruck minderwertiger Provenienz angesehen werden. Die Frühreife ging vielmehr auf Außenfaktoren zurück, die die Pflanzen in ihrer vegetativen Entwicklung gehemmt hatten.

Beobachtungen

Diese Feststellungen konnten in den folgenden Jahren an mehreren Beispielen erhärtet werden. So beobachteten wir, daß im Jahr nach der Depression des Höhenzuwachses wieder ein freudigeres Wachstum der geschädigten Lärchen zu verzeichnen war. Die folgenden Zahlen belegen dies. —

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

Gruppe	Anzahl	Zapfen	arithm. Mittel der Höhentriebe (in cm)			
		1958	*1955*	*1956*	*1957*	*1958*
A	12	10—50	29,5	46,2	17,4	71,2
B	8	10—50	39,5	26,1	13,8	52,3
C	7	—	28,5	37,-	16,3	66.8
D	4	—	26,-	27,5	43,-	87,-

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

Gruppe	Anzahl	Zapfen	arithm. Mittel der Höhentriebe (in cm)			
		1958	*1955*	*1956*	*1957*	*1958*
A	7	30—100	62,8	72,1	29,4	76,7
B	26	30—100	80,8	54,3	19,5	71,9

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

Der im Vergleich zu 1956 z. T. beträchtlich gesteigerte Höhenzuwachs von 1958 ist auch ein Ausdruck der günstigeren Wachstumsbedingungen, die in der Vegetationszeit von 1958 herrschten. Wir beobachteten ferner, daß eine nicht unerhebliche Anzahl Lärchen beider Anpflanzungen Zapfen trug, deren Zapfenspindel sich im Laufe des Sommers zu kleinen Zweigen ausgewachsen hatten, was — wie wir vermuten — ebenfalls mit der günstigen Sommerwitterung in Verbindung stand. Die aus den Zapfen hervorgewachsenen Zweige der Calvörder Lärchen *(Abb. 1)* waren bedeutend länger als die der Seehausener Lärchen, die nur wenige cm maßen. Obwohl in den beiden genannten Anpflanzungen etwa gleichgroße Höhentriebe für 1958 beobachtet wurden, zeigten sich doch erhebliche Unterschiede in der Zapfengröße. Diese betrug im Mittel von 100 Zapfen bei den Lärchen Calvörde = 2,1 cm, bei den Lärchen Seehausen dagegen 2,8 cm. Die Frequenz der Zapfenlängen ist in *Abbildung 2* dargestellt.

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