II.1 Maize × *Tripsacum* Hybridization and the Potential for Apomixis Transfer for Maize Improvement

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1 Introduction

Tripsacum, a genus related to maize, is found from the northern United States to southern Brazil. Divided into two sections, it includes 16 species (de Wet et al. 1976, 1981, 1983a; Brink and de Wet 1983; Doebley 1984), which occupy many ecologically distinct niches and habitats (Table 1). Most species are described as diploid or tetraploid, though a few have ecotypes at both ploidy levels. Meso-America is considered to be the center of diversity of the genus. Twelve of the 16 species mentioned in Table 1 occur in Mexico, and that is the main reason why an ORSTOM/CIMMYT *Tripsacum* program was initiated there.

Information on *Tripsacum* in general and on its potential for maize breeding in particular is still very limited. Maize × *Tripsacum* hybridization has been performed successfully for over 60 years, but so far, no one has demonstrated the potential of *Tripsacum* in maize improvement by transferring a useful agronomic trait from the wild relative to the cultivated crop. Since the first hybrids were produced by Mangelsdorf and Reeves (1931, 1939), many authors have documented the feasibility of crossing maize with *Tripsacum dactyloides*, which is widespread in the eastern USA (Randolph 1950; Maguire 1960; Anand and Leng 1963; Chaganti 1965; Galinat 1971, 1973; Simone and Hooker 1976; James 1981; Bernard and Jewell 1985; and others). Most of these studies, however, were aimed at elucidating relationships within the Maydeae and the origin of maize. Nonetheless, several investigators have shown, by backcrossing the F_1 intergeneric hybrids they produced and recovering "modified" 20-chromosome maize plants, that it is feasible to introgress *Tripsacum* traits into maize.

Our objective here is to show that all the information available from previous introgression attempts and cytogenetical studies make *Tripsacum* a very promising source of diversity for maize breeding, and justify new crossings, if these are based on new collections in Meso-America. Among other agronomical traits, emphasis is put on apomixis, as a potential tool to increase maize production in developing countries.

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Table 1. Ploidy levels and geographic distribution of *Tripsacum* species. (de Wet et al. 1976, 1981, 1983a; Brink and deWet 1983; Doebley 1984)

Species	Ploidy levels		Distribution
Section *Fasciculata*			
T. jalapense		4x	Meso-America
T. lanceolatum		4x	Northern Mexico, South Arizona
T laxum	2x		Tropical Meso-America
T. maizar	2x	4x	Tropical Meso-America
T. pilosum		4x	Tropical Meso-America
Section *Tripsacum*			
T. andersonii	3x + 10 (2n = 64)		Tropical America
T. australe	2x		South America
T. bravum	2x	4x	Central Mexico
T. cundinamarce	2x		South America
T. dactyloides	2x	4x	42°N to 24°S
T. floridanum	2x		Florida
T. intermedium		4x	Tropical Meso-America
T. latifolium	2x		Tropical Meso-America
T. manisuroides	2x		Chiapas, Mexico
T. peruvianum		4x 5x 6x	Peru, Ecuador
T. zopilotense	2x	4x	Central Mexico

2 Previous Introgression Attempts

Once maize × *Tripsacum* F_1 hybrids have been produced, three major backcross pathways have been followed in trying to introgress *Tripsacum* traits into maize. The first pathway, studied by Galinat (1971, 1973), started with a cross between a diploid maize (20Zm chromosomes) and a diploid *Tripsacum dactyloides* (36Td chromosomes). The F_1 hybrids had 2n = 28 chromosomes (10ZM + 18Td), and since the *Tripsacum* chromosomes could not pair at meiosis, 20-chromosome maize was recovered as soon as BC1, though the plants exhibited characteristics that were anything but maize-like (Fig. 1). Even so, this pathway was the first to allow production of addition lines combining the full set of maize chromosome plus an individual or pair of *Tripsacum* chromosomes. Less "buffered" than their wheat counterparts, these plants are more difficult to maintain. Nonetheless, they offer opportunities to study the effect of individual *Tripsacum* chromosomes, and in crosses with the appropriate B-A translocation, they may allow increased pairing between the *Tripsacum* chromosome and the part of the maize chromosome that has no copy to pair with (see Kindiger and Beckett 1990).

The second pathway, employed principally by Petrov in the USSR since the early 1960s (Petrov et al. 1979), started with a cross between a tetraploid maize (40Zm) and a tetraploid apomictic *T. dactyloides* (72Td). The F_1 hybrids had 2n = 56 chromosomes (20Zm + 36Td) and quite regular meiosis, since each chromosome had one homolog. In the BC1 generation, several plants had 2n = 38 chromosomes (20Zm + 18Td) and reproduced by apomixis. Further backcross generations led to the recovery of 20-chromosome maize, with morphological traits suggesting some kind of introgression. Apomixis was lost below

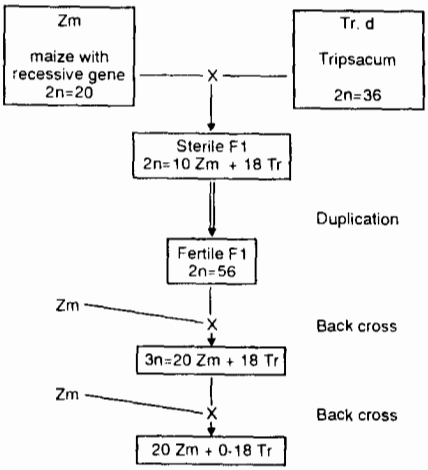

Fig. 1. Breeding chart illustrating the crosses involved in developing the various addition monosomic stocks of corn that carry extra cytogenetically identified chromosomes from *Tripsacum*. (After Galinat 1973)

the 38-chromosome level, because of a lack of adequate screening tools and a misunderstanding of the apomixis process as a whole.

In the Crop Evolution Laboratory at the University of Illinois, a third pathway was studied, which started with a cross between a diploid maize (20Zm) and a tetraploid *T. dactyloides* (72Td) (Harlan and de Wet 1977). The F_1 hybrids had 2n = 46 chromosomes (10Zm + 36Td), and it was possible to maintain this chromosome number for two or three consecutive generations, using a process referred to as "pseudoapomixis," since the ten chromosomes of maize were apparently replaced at each generation (Fig. 2). The same chromosome replacement has been observed at the University of Missouri (Beckett pers. commun.). Work at CIMMYT (Jewell pers. commun.) suggests that the same cross usually gives F_1 hybrids with 2n = 46 chromosomes, and these hybrids reproduce apomictically for several generations, i.e., without the ten maize chromosomes being replaced. Harlan and de Wet (1977) obtained a hybrid derivative with 2n = 56 chromosomes, probably from an unreduced gamete. Subsequent backcross generations permitted the recovery of a large number of maize plants with what the authors called "tripsacoid" morphological characteristics. Bergquist (1981) showed that some of these materials are resistant to certain corn diseases.

3 Cytogenetic Relationship Between Maize and *Tripsacum*

Mangelsdorf and Reeves observed early on (1935) that an F_1 hybrid of maize with a diploid *T. dactyloides* (2n = 36) has 28 unpaired chromosomes in meiosis, while the hybrid between maize and a tetraploid *T. dactyloides* (2n = 72) has 18 bivalents and 10 univalents. Since then, significant progress has been made in the

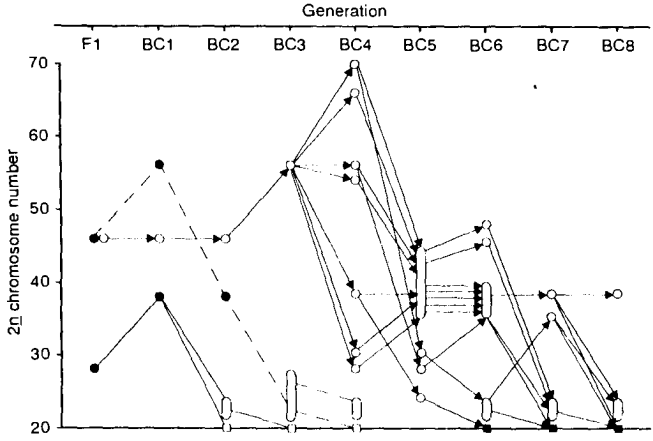

Fig. 2. Simplified diagram of some of the pathways to recovered maize from *Zea* × *Tripsacum* hybrids. The *stippled ovals* represent populations with the indicated range of chromosome numbers varying from plant to plant within families. □ Recovered maize (usually pure); ■ recovered maize (often tripsacoid); ● - ● 28 → 38 → 20 pathway; ● - ● 46 → 56 → 38 → 20 pathways; O → O irregular pathways (Harlan and de Wet 1977)

study of chromosomal relationships between maize and *T. dactyloides* (Maguire 1960; Galinat 1971; Rao and Galinat 1974, 1976). Several chromosomes of *Tripsacum* are shorter than those of maize, and the arm ratios of the two genera differ as well, with those of *Tripsacum* chromosomes usually ranging between 1:3 and 1:4 and those of maize between 1:1 and 1:2. In maize the nucleolus organizer is terminal in the short arm of chromosome 6, whereas in *Tripsacum* it may occur on two different chromosomes and is near the centromere in both cases (Randolph 1955; Tantravahi 1968). Homoeologies have been found between the chromosomes of *Tripsacum* and chromosomes 2, 4, 7, and 9 of maize. But loci common to both maize and *Tripsacum* have been identified on all but two maize chromosomes, as indicated in Table 2. Cytological markers, such as knobs, have usually – since Reeves and Mangelsdorf (1959) first made this observation – been found to be more numerous in *T. dactyloides* than in either maize or teosinte, and largely terminal in position. *Tripsacum*, like *Zea*, varies in knob numbers. Tantravahi (1968) reported that, although the North American species usually have many knobs, the Mexican species *T. maizar* has 0–3 knobs. This same author showed that *T. zopilotense* may be more variable. Other papers mention that the South American *T. australe* might be knobless (Graner and Addison 1944; Ting 1960). As Galinat (1971) showed, knobs may be useful for identifying chromosomes in interspecific hybrid derivatives, since knobs in *Tripsacum* are mostly terminal and occur on the long arms, while those of maize are mostly intercalary and occur on the short arm when they are terminal. This information from genetic and cytogenetic studies gives the impression that the relationship between maize and *Tripsacum* is somewhat loose. The use of new molecular tools

Table 2. Cytogenetic correspondance of some maize and *Tripsacum* chromosomes. (Galinat et al. 1970)

Chromosome no. in maize	Identified loci common to maize and *Tripsacum*	Morphology of the *Tripsacum* chromosome			
		Length (μ)	Arm ratio	Knobs no.	Assigned
M1	Bm_2				
M2 S	Ws Lg_1 Gl_2 b Sk Fl_1	22.3	1.7	TKL	Tr9
M2 L	V_4				Tr14 to 18
M3	A_1				
M4 S	Su_1 (but not La:Gl_3 Bm_3 Ra_3 J_2)	29.3	2.8		Tr7
M4 L	Gl_3 (but not La Su_1:Bm_3 Ra_3 J_2	22.4	3.5	TKL	Tr13
M5	Pr				
M7 S&L	O_2 V_5:Ra_1 Gl_1 I_1				(Tr4?)
M8	J_1				
M9 S&L	Yg_2 C Sh_1 Bz_1 Wx:Gl_{15} Bk_2 Bm_4	34.0	4.0	TKL	Tr5

will offer opportunities to further clarify this relationship. RFLPs are already being used at the University of Columbia, Missouri, and in our own *Tripsacum* program.

Other molecular markers have already been used, as in a study of *T. andersonii*, which was shown to have 2n = 64 chromosomes, with three genomes from a *Tripsacum* species and one from a *Zea* species (Levings et al. 1976; de Wet et al. 1983b). Talbert et al. (1990) recently pointed out the usefulness of transposable elements like Mu and Spm for clarifying such phylogeny. Their conclusions are questionable, however, since they tested only one plant accession from each putative parental species, even though these exhibit tremendous natural diversity.

Although *Zea* and *Tripsacum* have very different chromosome numbers, it has proved possible to cross the two, and backcrosses have been successful enough to recover 20-chromosome maize with new characteristics. Most studies in which this has been done, however, have focused on evolution and relationships within the Maydeae. The only real attempt to transfer an agronomic trait from *Tripsacum* to maize, made by Soviet scientists, was a failure.

4 Reasons for a New Attempt

4.1 Untouched Diversity

The only *Tripsacum* species that has been studied in relation to maize is *Tripsacum dactyloides*, or more precisely the part of the *T. dactyloides* diversity that is found in the USA. This species is found beyond the US-Mexican border, extending from the northern USA down to South America; there are at least 15 other described *Tripsacum* species; and, as mentioned previously, the center of diversity of the genus overlaps those of maize and teosinte in Mexico and

Guatemala. *Tripsacum* germplasm has been collected at different times by well-known scientists, including Hernandez X., Randolph, de Wet, Harlan, Timothy, and some of their students, but very little of this material has been used in research, apart from some taxonomic and evolutionary studies. This lack of interest has affected the quality of efforts to conserve the genetic resources already collected.

Since 1989, the ORSTOM/CIMMYT *Tripsacum* program has concentrated on making new collections. Over 1500 accessions have been assembled from 156 Mexican populations, found from sea level to 2500 m asl and from the driest habitats to some that remain humid all year round. The materials collected exhibit tremendous morphological diversity. New taxonomical analyses are underway which will make use of morphological and cytoembryological descriptors as well as biochemical markers.

From our observations and first cytological analyses, it seems that *Tripsacum dactyloides* is one component of a large agamic complex that could be used in maize breeding. This complex was defined more than 50 years ago by Babcock and Stebbins (1938), to characterize groups of plants that include sexual diploid types and apomictic polyploids (generally tetraploids). Although we discuss apomixis in a subsequent section of this chapter, it is worth mentioning here that this reproductive process is usually considered a nightmare for plant taxonomists, since it allows the survival and propagation of all possible intermediate (interspecific) morpho- and cytotypes. Normally, these would be eliminated by selection if they had originated from crosses between two sexual progenitors. The consequences of apomixis are well illustrated by the numerous triploids found so far in the populations we have collected, which reproduce by means of seeds. *Tripsacum* diversity appears to be continuous, making species definition very difficult. The variation of *T. dactyloides*, for example, is obviously continuous with that of *T. maizar* and *T. pilosum*, even though these species have been put in two different sections. Further analyses may show that this agamic complex is even more extensive than first suspected, and that other species must be included as well. The implication for maize × *Tripsacum* hybridization is that it should not be limited to the use of *dactyloides* species, as has been the case so far. This is supported by results from the first crosses we made at the end of 1990, which suggest that several species can easily be hybridized with maize (Table 3). In previous reports, the only candidate included has been *T. dactyloides* (Gutiérrez 1974), or if others were tested, backcrossing of these intergeneric hybrids was either unsuccessful or not even attempted. We used *T. dactyloides* accession # 65-1234, a tetraploid (2n = 72) from Florida, as a control, since this plant has long been successfully used in crosses at CIMMYT. We collected the hybrid embryos between 18 and 22 days after pollination, by which time almost all grains produced in the maize × 65-1234 crosses has a milky endosperm, suggesting that this combination is indeed a very special one and can probably result in the production of quite normal seeds. Another combination – maize × *T. intermedium* # 7159-3 – also gave grains with a milky endosperm, which might have given normal seeds had we had allowed this to happen. The accessions of *T. maizar* and *T. pilosum* that we were able to use in this preliminary experiment were also surprisingly productive.

Table 3. F_1 hybrid embryos produced in maize × *Tripsacum* crosses in September 1990 at CIMMYT. All cross-combinations made use of the same maize F_1 hybrid as pistillate progenitor

Pollinator	No. of maize cobs	No. of maize cobs with F_1 embryos	No. of F_1 embryos
T. dactyloides # 65-1234	32	24	732
T. dactyloides # 7127-6	15	4	15
T. dactyloides # 7139 ×	19	9	49
T. dactyloides # 7150 ×	8	8	88
T. dactyloides # 7156-1	4	3	67
T. dactyloides # 7203-x	39	27	218
T. intermedium # 7158-1	18	8	19
T. intermedium # 7159-3	23	15	103
T. maizar # 7207-3	14	5	83
T. pilosum # 7221-3	9	6	128
Total	181	109	1502

4.2 Better Technical Tools

When Petrov and his colleagues started their maize × *Tripsacum* program some 30 years ago, with the objective of transferring apomixis to maize, they were able to produce only six F_1 hybrids in two cycles (2 years). Our work in 1990 had only two objectives: (1) to train our team in crossing and embryo rescue techniques and (2) to identify possible problems. In January, 1993, we have ca. 1000 F_1 hybrids growing in the field, representing a wide array of intergeneric combinations, i.e. hybrids between maize and a series of different *Tripsacum* species. Obviously, the embryo rescue techniques used in the 1970s and improved during recent years (Bernard and Jewell 1985; Furini and Jewell, pers. commun.) are of major importance in recovering such wide cross hybrids. Somatic embryogenesis (Furini and Jewell 1991) may be also extremely useful, since most F_1 hybrids show complete sterility. The very few that exhibit some degree of fertility could be multiplied in this way, permitting the production of larger BC1 populations. ca. 5000 BC1s are now being studied. Other new techniques may also be employed, including pollen conservation at –80 °C. We have met with success in testing Hanna's technique (Hanna 1990). Six months after collection, the pollen is still viable. Drying conditions other than those used by Hanna are currently being tested and are better adapted to conditions at our research station in the tropics, which is quite far from our laboratory. More effective conservation of pollen, in addition to allowing us to make crosses that would otherwise be impossible because of differences in flowering periods, may help us with the difficult task of producing large numbers of BC1 hybrids. When apomixis is transferred, as discussed later, the F_1 hybrids and hybrid derivatives, when apomictic, can only be used as pollinators. Thus, even if maize × *Tripsacum* F_1 hybrids look 100% male sterile, enough pollen grains have to be produced to break through the BC1 generation. If low temperature conservation allows us to collect anthers whenever the F_1 hybrid flowers, and to keep them until enough pollen from the same F_1 hybrid combination is available to be put on maize, then we might expect to achieve some efficiency in producing more than the very few BC1 plants that

usually result from this type of wide cross. Another advantage of in vitro culture for obtaining large numbers of F_1 and BC1 progenies is that we can culture these plants in tubes for quite a long time, getting quite vigorous seedlings to better ensure their regrowth after transplanting in pots.

4.3 A Useful Trait to Transfer

In 1974 Gutièrrez pointed out that species of *Tripsacum* are adapted to a wide range of environmental conditions and thus constitute a vast reservoir of potentially valuable genes for maize improvement. The traits he listed are as follows:

1. Exceptional tolerance to differences in day-length and extremes of temperatures.
2. Wide range of adaptation to various kinds of soils.
3. Cold tolerance.
4. An essentially disease-free root system.
5. Resistances to most corn diseases.
6. Rapid post-fertilization seed maturity.
7. Exceptional hybrid vigor potential, as seen among various *Tripsacum* species hybrids.

To this list we could probably add:

8. Resistance to most corn pests.
9. Protein quality.
10. Apomixis.

Since most of these traits are quantitative, not much could be accomplished through a conventional backcross program in transferring them to maize. The development of RFLP maps for maize and its relatives, however, and recent progress in mapping QTLs have opened up new possibilities in screening for such traits in successive backcross generations. As mentioned previously, RFLPs are already being used in work on *Tripsacum* at the University of Missouri (Blakey et. al. 1990), as well as in our own program in Mexico.

However, the major reason for new efforts in maize × *Tripsacum* hybridization is that we now know of an extremely valuable agronomic trait – apomixis – which fits the minimum conditions for such an intergeneric transfer. The trait is genetically controlled, probably by no more than a single dominant gene. It is also easy to identify and screen in the progenies.

5 Manipulation of Apomixis

Apomixis was first described in *Tripsacum dactyloides* by Farquharson in 1955. A recent study, with accessions of the same species (Burson et al. 1990), showed that, while the diploids are sexual, triploids and tetraploid accessions may

reproduce by diplospory. This type of diplospory is similar to the type described in *Elymus rectisetus*, a relative of wheat (Crane and Carman 1987). Meiosis does not follow the normal sequence of events, with most of the prophase being replaced by a long resting stage, followed by a mitotic-like division and then production of an unreduced gamete that is genetically identical to any cell of the maternal tissues. The embryo later develops without fertilization, producing offspring that are genetically maternal.

5.1 Why Apomixis?

Apomixis offers plant breeders many advantages (Hanna and Bashaw 1987). First, it greatly simplifies the selection of promising genotypes; any interesting combination that reproduces by apomixis is permanently fixed and will breed true from one generation to the next without complications. Second, apomixis allows many combinations to survive that would not be fertile if they were sexual, and can thus provide the breeder with a much larger genetic base for seeking hybrid vigor. Third, it facilitates the production of hybrid seed by eliminating the need for growing different progenitors in isolated fields and for costly manual operations; apomictic hybrid seed could be harvested from the parent in much the same way that hybrid production is harvested today.

The main advantage of apomixis, however, applies to farmers in the Third World, most of whom still have not adopted improved maize varieties. In Mexico, for example, it is estimated that 75% of the maize area is sown to farmers' own seed, and the proportion is even higher (90%) in West Africa. In this latter region, the Green Revolution approach might work in selected areas but in general would require imported inputs, along with financial support and technical assistance. Unlike traditional agriculture, this approach is highly subject to external shocks, such as increases in the price of oil or the withdrawal of international assistance, and is therefore impossible to implement under environmental conditions as limiting as those in West Africa's Sahel. Other approaches must be developed to better enable small farmers to achieve a secure food supply. Apomixis may just be the simple tool that farmers need to increase production significantly without major changes in their production practices. Suppose, for example, that apomixis can be transferred to a population cultivated by a given farmer. When he chooses 50 good ears to provide seed for the next cylce, he will have 50 different genotypes that are fixed in all their characteristics. In this seed he will save the genetic heterogeneity he needs to secure his harvest against most environmental pressures (including pests and diseases). Moreover, all plants will give good ears, and overall production will be better than ever. Farmers are unlikely to achieve food self-sufficiency if only 5% of them can raise their yields to 4 t/ha, but they could probably produce food surpluses if average production *on all farms* could be increased even modestly from the current 1.2–1.3 t/ha to 1.6t/ha in the case of maize. It is thus highly worthwhile to search for practical solutions that benefit all small farmers. Apomixis appears to be such a solution, but introducing it into maize will be possible only if genetic control of the trait is very simple.

5.2 Genetic Control of Apomixis

Many genetic analyses have been published, and these are mentioned in a review on apomixis by Nogler (1984a). Only three studies, however, made use of adequate germplasm, including sexual diploids found in the wild. The rest were largely inconclusive for various reasons, including the progenitors used, the number of progenies studies, and the screening tools employed. Table 2 summarizes the data collected by Harlan et al. (1964) on the *Bothriochloa-Dichanthium* complex and by Savidan (1981, 1982) and Savidan et al. (1989) on *Panicum maximum*, and Fig. 3 presents the findings of Nogler (1984b) on *Ranunculus*. Although the two tropical forage grasses have nothing in common with the small Ranunculaceae, all of the data available fit perfectly the same genetic model, namely that apomixis is controlled by no more than one dominant gene.

Other analyses have been made in other grasses, including *Paspalum* (Burton and Forbes 1960), *Cenchrus* (Taliaferro and Bashaw 1966; Read and Bashaw, 1969), *Eragrostis* (Voigt and Bashaw 1972), and *Panicum* (Hanna et al. 1973). Although these authors usually state that apomixis is simply inherited, the model they propose is generally di- or trigenic. The only point these analyses have in common is that they can be explained by models other than those proposed by the authors; the data are simply insufficient to be conclusive. Some of the analyses have been repeated, e.g., that of *Panicum* (see Table 4) or are being repeated, as for *Paspalum* (Quarin and Urbani pers. commun. 1987) and give segregations that all fit the model of one dominant gene. Another analysis, underway in Brazil and at CIAT (Centro Internacional de Agricultura Tropical), Colombia, focuses on the genus *Brachiaria* and also gives segregations that agree with the model of one dominant gene.

Table 4. Segregations observed in two genetic analyses, made on the *Bothriochloa-Dichanthium* complex (Harlan et al. 1964) and on *Panicum maximum*. (Savidan 1981, 1982)

Cross-combination types	Observed		Expected from the model	
	apo	sex	apo	sex
Bothriochloa-Dichanthium				
1. sexual 4x × sexual 4x	0	40	0	40
2. apo × sex and sex × apo	58	19	63	14
3. apo × apo	74	5	75	4
Panicum maximum				
1. sexual 4x × apo1	71	62	67	66
2. 3way sex(S1×A1) × A2	135	144	139	140
3. sexF1 selfed	0	126	0	126
4. sex3way selfed	0	57	0	57
5. BC: sex (S1×A1) × A1	14	12	13	13
6. BC: sex3way × A2	73	97[a]	85	85
7. FS: sex × sex	0	82	0	82
8. FS: sex × apo	26	34	30	30
9. TC: S1 × apo(S1×A1)	13	10	12	11
10. apo × apo	53	18	53	18

[a] Undetected selfed progenies were included.

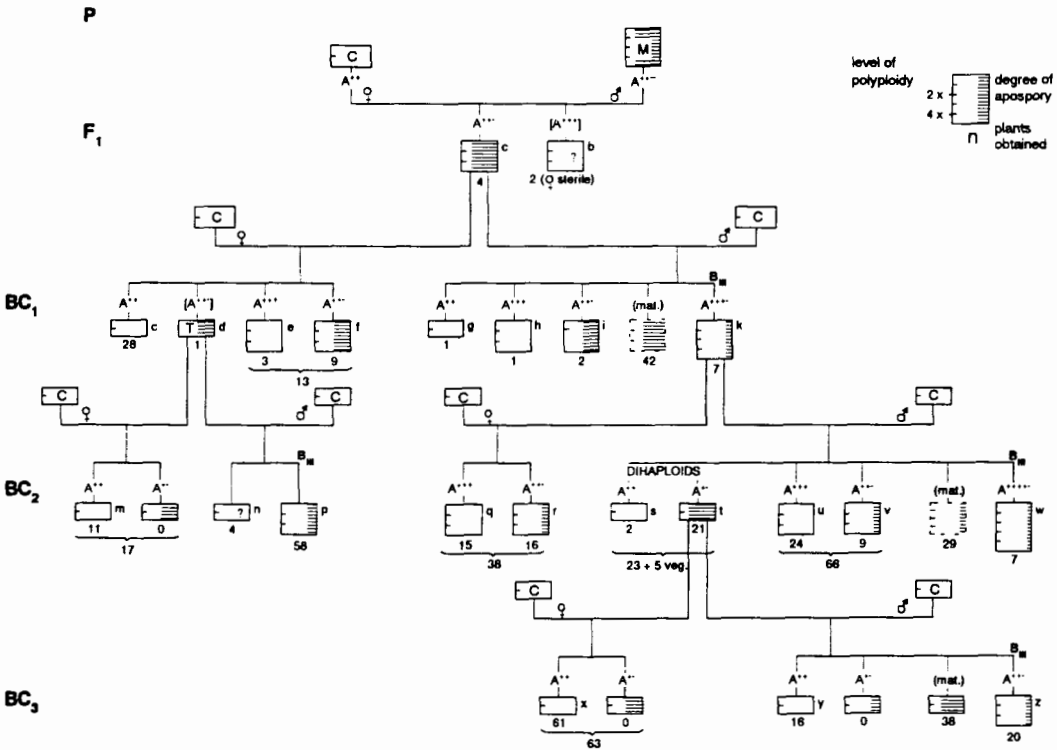

Fig. 3. Genealogical tree of the crossing *Ranunculus cassubicifolius* = C, 2n = 16, meiotic (sexual) × *R. megacarpus* = M, 4x = 32, partially aposporous ("totally" apomictic) and the different backcrossings with the sexual parent C. The number of plants obtained, the polyploidy level, the approximate degree of apospory, and the genotype are indicated for each offspring. (Nogler 1984b)

In recent studies at Utah State University (Crane and Carman 1987; Carman et al. 1991), the first observations have been made of what may be the effect of the apomixis gene. In *Elymus scabrus*, as in other sexual species, the megaspore mother cells (MMCs) are isolated prior to meiosis by a thick wall of callose. Apomictic accessions of *E. rectisetus*, which belongs to the same agamic complex as *E. scabrus*, do not have this callose wall around their MMCs. This may allow molecules to diffuse from the MMCs to surrounding cells, with the result that these MMCs can no longer undergo normal meiosis and/or that surrounding somatic cells of the nucellus acquire the ability to produce an embryo sac, a function that is normally reserved for the megaspore. The studies at Utah continue, and similar ones are being started under the auspices of APONET[2].

5.3 Screening Tools for Transfer of Apomixis to Maize

Although Burson et al. (1990) used a somewhat tedious, conventional embryological technique, like that employed in the earliest studies, new clearing techniques can be used to study apomixis in *Tripsacum*. In a recent comparison of the techniques available, we found that methyl salicylate, as used by Young et al. (1979), was by far the most satisfactory clearing solution. Herr and Crane's solutions (Herr 1982; Crane and Carman 1987) also cleared perfectly the materials under study but did not perform as well under interference contrast microscopy. From preliminary observations, it seems that the triploid, tetraploid, or hexaploid cytotypes of all *Tripsacum* species reproduce by apomixis, in which diplospory is followed by parthenogenesis. The diploids appear to be sexual. If confirmed, these first observations will fit the general concept of the agamic complex, as defined by Babcock and Stebbins (1938).

6 Conclusions

Maize cytogenetics is far from having contributed as much as wheat cytogenetics to crop improvement. It seems much easier to manipulate alien material in wheat breeding than in maize. However, this does not justify the relative lack of interest in *Tripsacum* diversity and its potentially beneficial agronomic traits, especially when one considers the technical advances achieved in the last two or three decades. It is now possible to make maize × *Tripsacum* F_1 hybrids by the thousands. The sterility of the first generations can be overcome with a series of new tools, and it will take just six to eight generations (or at most 10 years) to arrive at a 20-chromosome plant bearing traits transferred from the wild species,

[2] APONET, International Network for Apomixis Research has been recently set up, and is coordinated by the senior author. One program of the network, which includes the participation of several labs in Europe and Latin America, intends to study the effect of the apomixis gene and prepare for its manipulation in genetic engineering.

assuming that we end this story by using B-A translocations to force recombination between maize and *Tripsacum* chromosomes.

Apomixis has been identified in African grasses and is present in the genus *Tripsacum*. It could become a major tool for development in Third World countries. Recent studies show that this trait is simply inherited and can be easily manipulated through backcrossing. The ORSTOM/CIMMYT *Tripsacum* program, while looking for other important agronomic traits, such as insect and other stress resistances, will focus on the transfer of apomixis into maize. By the end of the 1990s, these efforts should result in the release of the first apomictic maize germplasm.

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