Theor Appl Genet (1995) 90: 1198–1203


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# Detection of the apomictic mode of reproduction in maize-*Tripsacum* hybrids using maize RFLP markers

Received: 22 December 1994 / Accepted: 17 February 1995

**Abstract** Polyploid plants in the genus *Tripsacum*, a wild relative of maize, reproduce through gametophytic apomixis of the diplosporous type, an asexual mode of reproduction through seed. Moving gene(s) responsible for the apomictic trait into crop plants would open new areas in plant breeding and agriculture. Efforts to transfer apomixis from *Tripsacum* into maize at CIMMYT resulted in numerous intergeneric $F_1$ hybrids obtained from various *Tripsacum* species. A bulk-segregant analysis was carried out to identify molecular markers linked to diplospory in *T. dactyloides*. This was possible because of numerous genome similarities among related species in the Andropogoneae. On the basis of maize RFLP probes, three restriction fragments co-segregating with diplospory were identified in one maize-*Tripsacum dactyloides* $F_1$ population that segregated 1:1 for the mode of reproduction. The markers were also found to be linked in the maize RFLP map, on the distal end of the long arm of chromosome 6. These results support a simple inheritance of diplospory in *Tripsacum*. Manipulation of the mode of reproduction in maize-*Tripsacum* backcross generations, and implications for the transfer of apomixis into maize, are discussed.

**Key words** Diplospory · RFLP · Bulk-segregant analysis · Genome similarity · Intergeneric hybrids · *Zea mays*

Communicated by G. S. Khush

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## Introduction

Barring a few exceptions, such as banana and potato, important food crops reproduce sexually. By contrast, apomixis–asexual reproduction through seed (Nogler 1984a) is a very common mode of reproduction in perennial tropical forage grasses and in at least 300 wild species belonging to 30 higher plant families (Asker and Jerling 1992). Apomictic processes bypass both meiosis (apomeiosis, after Renner 1916) and egg-cell fertilization, producing offspring which are exact genetic replicas of the mother plant. Particular genotypes or complex traits as well as hybrid vigor, impossible or difficult to perpetuate through sexual reproduction, can be easily maintained and multiplied indefinitely under an apomictic mode of reproduction. Therefore apomixis would be of great value if introduced into major food crops, and would allow the propagation of heterotic hybrid combinations, leading to increased food production in developing countries (Savidan and Dujardin 1992; Jefferson 1994) . Most major cereal crops belong to the Gramineae, a family in which apomixis is widely expressed (Brown and Emery 1958), especially in wild relatives of maize, wheat, and pearl millet. However, owing to the wealth of cytological and molecular data available for maize, combined with the recent progress in understanding its reproductive biology (Dumas and Mogensen 1993; Faure et al. 1994) , maize would be a unique model system for the manipulation of apomixis.

The mode of reproduction in the *Tripsacum* agamic complex (x=18), the closest wild apomictic relative of *Zea mays* L. (x=10), is closely related to ploidy level. Diploid genotypes are sexual, but polyploid ones reproduce through gametophytic apomixis characterized by diplosporous apomeiosis (diplospory) followed by parthenogenetic development of the egg cell. Diplospory in *Tripsacum* ssp. is facultative and primarily of the *Antennaria* type, resulting from meiotic failure in megasporocytes that first enlarge and then directly develop into mature unreduced female gametophytes through three or more mitoses (Leblanc et al. 1995). Moreover, non-reduction of chromo-

somes during diplosporous megasporogenesis – i.e. meiosis failure – is strongly associated with an absence of callose deposition in megasporocyte cell walls, whereas meiotic megasporocytes and their derivatives are surrounded by callose (Leblanc et al. 1995). In our current effort to transfer apomixis from *Tripsacum* into maize, $F_1$ hybrids were produced using maize and tetraploid *Tripsacum* accessions from various origins. We have used one $F_1$ population that segregated 1:1 for mode of reproduction (meiotic vs apomeiotic), together with bulk-segregant analysis (BSA; Michelmore et al. 1991) for the detection of genetic markers linked to gene(s) responsible for apomeiosis in *Tripsacum*. Taking advantage of the genomic synteny observed within the Andropogoneae (Bennetzen and Freeling 1993), a strategy based on molecular markers of known map position in maize was devised to screen the *Tripsacum* DNA bulks. The molecular-marker information reported here should assist in the transfer of apomixis from *Tripsacum* into maize, as well as ultimately allow the isolation of the *Tripsacum* DNA segment responsible for diplospory for more detailed molecular analyses.

## Materials and methods

### Plant material

An $F_1$ population of 91 individuals was derived from hybridization between a maize hybrid (CML139 × CML135) from Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) as pistillate parent and *Tripsacum dactyloides*(L.) L., CIMMYT accession 65-1234 (2n=4x=72), an apomict from the Everglades, Florida, USA (Leblanc et al. 1995) . All the hybrids recovered were 2n=46 combining 36 chromosomes from the *Tripsacum* parent and 10 from the maize parent. They were all completely male-sterile, and only a few showed very low female fertility when backcrossed to maize. Given such fertility behavior, the determination of the mode of reproduction of each $F_1$ plant through progeny-tests (i.e. mother-offspring comparisons and/or evaluation of progeny heterogeneity) could not be carried out. Therefore, analysis of callose deposition during megasporogenesis (sucrose-aniline blue clearing procedure combined with epifluorescence microscopy; Peel 1993) was used for cytoembryological determination of reproductive behavior (meiosis versus diplospory) in 52 maize-*T. dactyloides* hybrids. Some 10 to 20 ovules from 31 of the 52 hybrids previously analyzed were then re-cleared using a methyl benzoate-dibutyl phthalate clearing procedure (Crane and Carman 1987) to confirm callose analysis on the basis of cytological observations.

### Bulk DNA composition and RFLP analysis

DNAs from 53 individuals of the maize-*Tripsacum* $F_1$ population and the two parents were extracted using a CTAB procedure (Hoisington et al. 1994). For bulk-segregant analysis (BSA), two bulks of DNA were prepared by combining the DNA from ten $F_1$ hybrids producing reduced (meiotic) female gametophytes, and from 10 $F_1$s producing unreduced (apomeiotic) female gametophytes. DNA from $F_1$ hybrids, the maize and *T. dactyloides* parents, and the two bulks, were digested using the restriction endonucleases *Hin*dIII and *Eco*RI, electrophoresed and transferred onto nylon membranes. Hybridizations, stringency washes (0.15 × SSC-0.1% SDS, 2 × 5 min. at room temperature, then 3 × 15 min at 60°C), chemiluminescent detection, and exposure were all performed according to Hoisington et al. (1994).

Maize RFLP clones (BNL, CSU, UMC and php) were kindly supplied by the University of Missouri, Columbia. The final selection of probes was based on the preliminary screening of five $F_1$ individuals and both the maize and the *Tripsacum* parents, in order to identify *Tripsacum*-specific restriction fragments as well as heterozygosity for these fragments in the *Tripsacum* parent. For the genetic study, the DNAs of *T. dactyloides* (accession 65-1234), the maize hybrid (CML139 × CML135), and the two bulks, were hybridized to 93 clones detecting loci throughout the maize genome (at 20 to 40 cM intervals; Heredia Díaz et al. personal communication; tropical maize map, CIMMYT, unpublished data). *Tripsacum*-specific markers identified in the diplosporous bulk were then checked on the 44 $F_1$ individuals of known reproductive behavior. Once markers had been identified, eight probes corresponding to other flanking markers on the maize map, were then used to narrow down the region of interest.

### Isozyme analysis

Isozyme analysis was performed following Stuber et al. (1988) , after slight modification of the sample preparation procedure; samples were extracted by homogenizing 0.2 g of young leaves from adult plants with 200 µl of a Tris-HCl 0.5 M pH 7.5 buffer containing 16% (w/v) sucrose, 8% (w/v) sodium ascorbate, 8% (w/v) PVP and 4% (w/v) ascorbic acid. $F_1$ hybrids in bulks and the two parents were stained for MDH (malate dehydrogenase).

## Results

### Segregation for mode of reproduction in the maize-*Tripsacum* $F_1$ hybrids

Of the 52 maize-*Tripsacum* $F_1$ hybrids analyzed for mode of reproduction, 23 lacked callose deposition during megasporogenesis and 29 exhibited callose fluorescence in and around the megasporocyte or the megaspore cell walls. When observed after the methyl benzoate-dibutyl phthalate clearing procedure, all the ovules showing an absence of callose revealed enlarged megasporocytes of the *Antennaria* diplosporous type, whereas the fluorescing ones always contained meiotic products (megasporocytes, dyads or tetrads). These observations correspond to typical diplosporous gametophytic apomixis and sexuality in *Tripsacum* ssp. (Leblanc et al. 1995). Several different hypotheses were tested regarding the segregation ratio for mode of reproduction; three of these are given on Table 1.

**Table 1** Possible hypotheses pertaining to the segregation of the mode of reproduction in the *T. dactyloides*-maize segregating $F_1$ population. Each of the expected ratios can be fitted to the observed segregation of 23 (diplosporous) to 29 (meiotic); r is the recombination fraction and *P* the probability associated with the test

| Hypothesis | Expected ratio | $\chi^2$ | *P* |
|---|---|---|---|
| One factor | 1 : 1 | 0.7 | $0.5 > P > 0.25$ |
| Two independent factors | 3 : 1 | 10.3 | $0.005 > P$ |
| Two factors linked (r=0.25) | 54 : 46 | 0.065 | $0.9 > P > 0.75$ |

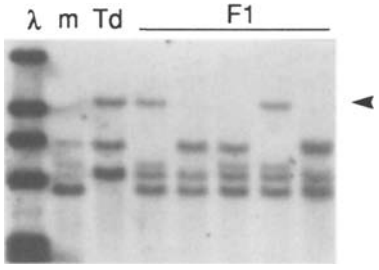


**Fig. 1** Hybridization of maize probe CSU4 onto DNA of *T. dactyloides* 65-1234 (*Td*), the maize hybrid CML139×CML135 (*m*), and five $F_1$ individuals. The *arrow* indicates a *Tripsacum*-specific restriction fragment. Note that the *Tripsacum* parent is heterozygous for this fragment. λ *Hin*dIII-digested lambda DNA

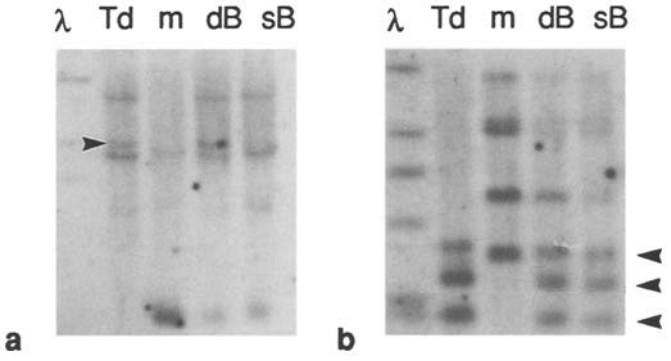


**Fig. 2a, b** RFLP patterns in *T. dactyloides* (65-1234) (*Td*), the maize hybrid (CML139×CML135) (*m*), and the two $F_1$ DNA bulks (*dB* diplosporous DNA bulk; *sB* sexual DNA bulk) revealed by two maize probes. **a** Polymorphic restriction fragment (*arrow*) shared by *T. dactyloides* 65-1234 and the diplosporous bulk detected by UMC28 (*Eco*RI digests). **b** *Tripsacum*-specific restriction fragments (*arrows*) that were not correlated with the mode of reproduction detected by BNL6.22 (*Hin*dIII digests). λ *Hin*dIII-digested lambda DNA

## RFLP analysis

Eighty-four out of ninety-three maize probes (90%) detecting loci throughout the maize genome, revealed *Tripsacum*-specific restriction fragments. Among these probes, all the "CSU" gave the strongest and clearest signals when hybridized onto *Tripsacum* DNA; these cDNA clones seemed therefore to have better homology to *Tripsacum* DNA than most of the others which were derived from genomic libraries. The inheritance of the *Tripsacum*-specific restriction fragments in the preliminary probe selection screening of five $F_1$ individuals showed that 81% were heterozygous in the *Tripsacum* parent (Fig. 1). The two restriction enzymes used (*Eco*RI and *Hin*dIII) were equally effective in detecting informative polymorphisms. The final 68 maize probes (Table 2) were hybridized to *T. dactyloides* (accession 65-1234), maize hybrid (CML139 × CML135), and the two DNA bulks.

**Table 2** RFLP probes detecting loci throughout the maize genome (20–40-cM intervals), selected for RFLP analysis

| Maize chr. | Probes detecting a locus in a given chromosome[a] |
|---|---|
| 1 | BNL 5.59, BNL 6.32, UMC11, UMC58, UMC72, UMC83, UMC86, CSU3, CSU12[b], CSU110, CSU134. |
| 2 | BNL8.45, UMC36, UMC55, UMC98, UMC139, CSU4, CSU40, CSU64, CSU154. |
| 3 | UMC16, UMC17, UMC18, UMC92, CSU32, CSU38. |
| 4 | BNL8.23, UMC47, UMC66, CSU39, CSU84, CSU166. |
| 5 | BNL6.22, UMC1, UMC54, UMC68, CSU26, CSU33, CSU108. |
| 6 | UMC28, UMC65, UMC138, CSU56, CSU80, CSU70 CSU94. |
| 7 | BNL5.61, UMC80, UMC149, CSU11, CSU27, CSU129. |
| 8 | BNL1.45, UMC120, CSU31, CSU110, CSU142. |
| 9 | UMC105, UMC114, CSU12[b], CSU93, CSU95. |
| 10 | BNL7.49, UMC64, UMC130,CSU6, CSU46, CSU103. |

[a] Heredia Díaz et al. pers. com.; tropical maize map, CIMMYT, unpublished data
[b] Probe for which duplicate loci are known in the maps mentioned above

**Table 3** Sub-set of genetic markers from a distal segment of maize chromosome 6 used for the detection of linkage(s) with the chromosome region controlling diplospory in *Tripsacum*

| Probe | Map distance (cM) from UMC28[a] |
|---|---|
| UMC177[b] | 3.4 |
| CSU68 | 0 |
| UMC134 | 1.5 |
| MDH2 | 9.7 |
| UMC133 | 9.7 |
| php4016 | 15.0 |
| UMC62 | 17.7 |
| UMC71 | 24.7 |
| UMC132 | 30.8 |

[a] Distances are based on the 1994 map of Heredia Díaz et al. (personal communication)
[b] Most-distal locus known

## Linkage analysis

The screening of DNA bulks yielded three potential markers for the mode of reproduction. The first marker was detected by UMC28 during the initial screening of the bulks. Further analysis of the probable flanking regions, based on the maize map (Table 3), yielded two more markers, detected by CSU68 and UMC62. In effect, these probes each detected a restriction fragment present in both the *Tripsacum* parent and the diplosporous bulk, but not in the other bulk or the maize parent (Fig. 2). After hybridizing the corresponding probes to the 44 individuals of known mode of reproduction, significant linkage between all three markers and diplospory was detected (Table 4 and Fig. 3). All three single dose markers segregated according to a 1:1

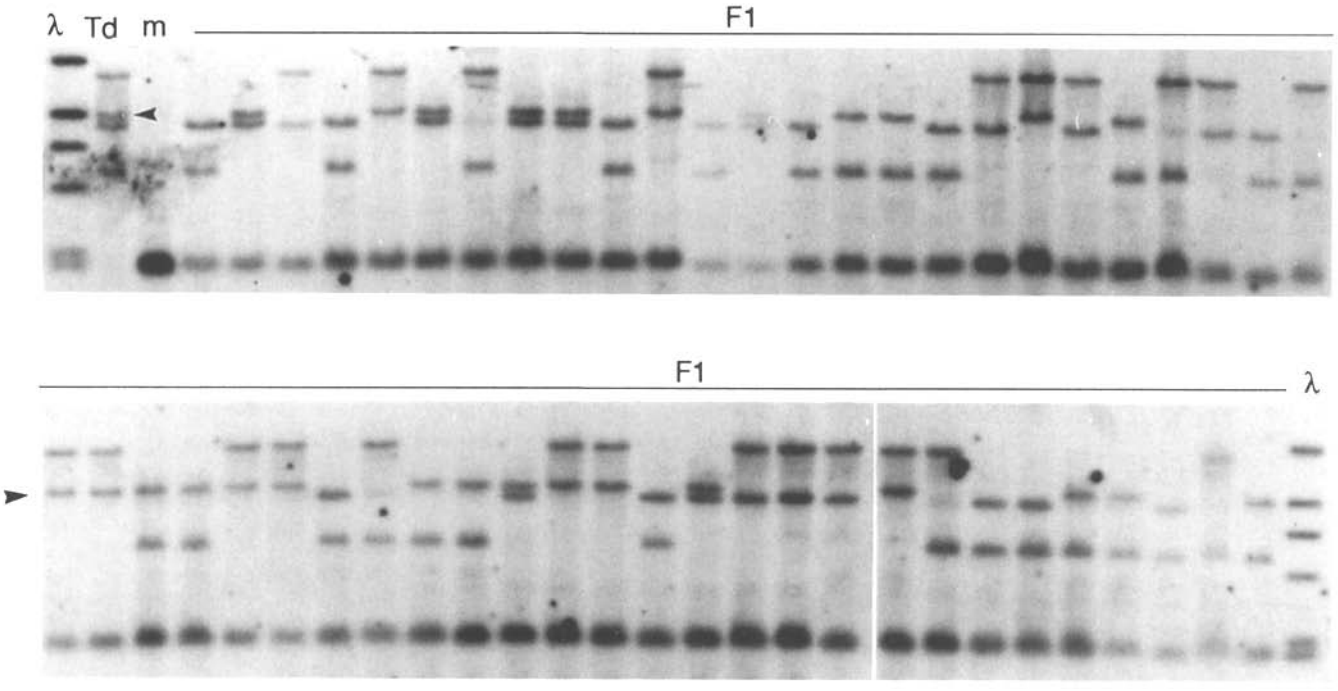


**Fig. 3** Segregation analysis of the restriction fragment revealed by UMC28 (*arrow*) in the $F_1$ hybrids from the cross between diplosporous *T. dactyloides* (*Td*) and sexual maize (*m*). *Eco*RI digests. λ *Hin*dIII-digested lambda DNA

**Table 4** Linkage relationships between the three RFLP markers and diplospory detected through BSA, in the segregating $F_1$ population of maize hybrid (CML139 × CML135) × *T. dactyloides* (accession 65-1234). Each cell contains the results of the contingency $\chi^2$ test for independent assortment; *P* is the probability associated with the test and r is the recombinant fraction

| Marker | Diplospory | Marker | |
|---|---|---|---|
| | | umc28 | csu68 |
| umc28 | $\chi^2 = 17.8$<br>$P = 2.5 \times 10^{-5}$<br>r = 0.18 | | |
| csu68 | $\chi^2 = 17.8$<br>$P = 2.5 \times 10^{-5}$<br>r = 0.18 | $\chi^2 = 44.0$<br>$P = 9.3 \times 10^{-7}$<br>r = 0.00 | |
| umc62 | $\chi^2 = 19.8$<br>$P = 8.7 \times 10^{-6}$<br>r = 0.15 | $\chi^2 = 29.8$<br>$P = 4.3 \times 10^{-7}$<br>r = 0.05 | $\chi^2 = 29.8$<br>$P = 4.3 \times 10^{-7}$<br>r = 0.05 |

ratio ($\chi^2 \leq 0.27$, $P \leq 0.6$). There were no recombinants between the markers detected by UMC28 and CSU 68, irrespective of the enzyme used.

## Discussion

### Inheritance of diplospory

Some of the hypotheses assuming the more complex models of genetic control of diplospory (Table 1) are too improbable. As will be discussed later, the marker data seem to support the hypothesis that the inheritance of diplospory in *Tripsacum* is controlled by a single Mendelian factor. Under this hypothesis, tetraploid *T. dactyloides* would be simplex for a dominant allele conferring diplospory.

Regarding the genetic control of diplospory in other species, no genetic analysis has been conducted, apart from studies in *Taraxacum* which show that the gene or genes for diplospory are located on a single chromosome (Mogie 1988).

In the case of apospory, where one or more unreduced female gametophytes form mitotically from somatic nucellar cells while the legitimate sexual line generally aborts, similar results have been obtained for various species. The inheritance of gametophytic aposporous apomixis appears to be monogenic in *Panicum maximum* (Savidan 1982), *Ranunculus auricomus* (Nogler 1984b), *Cenchrus ciliaris* (Sherwood et al. 1994), and *Brachiaria* (Valle and Miles 1994) .

### Strategy for DNA bulk screening: RFLP vs RAPD markers

In our endeavor to tag genes for diplospory, two crucial aspects of our strategy were the use of existing RFLP linkage information for maize and the speed provided by bulk-segregant analysis. BSA is now commonly performed in molecular genetic studies for the identification of markers linked to traits of agronomic value. RAPD markers are mainly used in such analyses (e.g., Burua et al. 1993; Penner et al. 1993), but note that bulk screenings are usually performed in the absence of a linkage map that is valid for any segregating population of the species concerned. On the other hand, although RFLP markers have been widely

used for constructing maps that are valid across populations of a species (e.g., the maize maps of Helentjaris et al. 1986; Burr et al. 1988; Gardiner et al. 1993), they are only sporadically used in bulk-segregant analyses (e.g., Pineda et al. 1993; Mohan et al. 1994). Over the last 10 years, grass genomes have been well characterized genetically, and molecular data have revealed important similarities (gene order and composition, map collinearity) within and between the Triticeae and the Andropogoneae (Hulbert et al. 1990; Liu et al. 1992; Ahn et al. 1993; Melake Berhan et al. 1993; Grivet et al. 1994). Although a genetic linkage map is not available for *Tripsacum*, preliminary results from our group indicate that regions/groups of markers are conserved between the maize and *Tripsacum* genomes (unpublished data), and confirm Galinat's (1971) previous cytogenetic work. Because of such genomic similarities, the selected set of probes allowed us to use the maize molecular linkage map as a basis for screening efficiently the*Tripsacum* linkage groups.

Moreover, as expected in a wild apomict (Nogler 1984a), a very high level of heterozygosity was detected in the parental *Tripsacum* accession. This and the large number of *T. dactyloides*-specific restriction fragments, facilitated the discovery of markers co-segregating with diplospory after a relatively modest screening.

## Linkage between molecular markers and diplospory

The three RFLP markers found to co-segregate with diplospory re-inforce the hypothesis of monogenic inheritance of diplospory. All three markers also appear to belong to the same linkage group in maize: the distal end of the long arm of chromosome 6 (Heredia Díaz; CIMMYT *loc cit*). This observation confirms the existence of conserved genomic segments between *Tripsacum* and maize. Moreover, the recombination observed between the loci detected by UMC28 (or CSU68) and UMC62 was lower (0.05) in *Tripsacum* than in the available maize maps (around 0.2, Heredia Díaz; CIMMYT *loc cit*). The significance of this difference is difficult to interpret as yet. Further analysis using more markers and progenies from both tetraploid and diploid *Tripsacum* crosses should indicate whether there are significant differences in the recombination rates between the two ploidy levels and maize. The most relevant result, however, is that the segments may be homologous enough for recombination to take place between maize and *Tripsacum* genomes around the region controlling diplospory.

Co-inheritance of apomixis and molecular markers has been previously reported in *Pennisetum squamulatum* Fresen, a wild relative of pearl millet (*Pennisetum glaucum* L.) that reproduces through aposporous apomixis (Ozias-Akins et al. 1993). However, as observed by these authors, Mendelian linkage analysis could not be performed on this type of plant material since there was no evidence of pairing between the putative chromosome carrying the factor(s) for apospory and a chromosome from the other parent. In such a system the prospects for applying marker-assisted selection to introduce apomixis to a recipient species seem daunting. In contrast, our current data suggest that the apparent homoeology between *Tripsacum* and maize could favour chromosome pairing between the corresponding segments and hence the possibility for recombination to occur. The three RFLP markers (UMC28, UMC62 and CSU68) that co-segregate with diplospory in *T. dactyloides* could well represent a first step in assisting the selection of such recombination events which are necessary for the ultimate production of diplosporous maize.

The question remains of whether the two essential components of apomictic reproduction, in this case diplospory followed by parthenogenesis, are determined by independent or linked genetic factors. Indeed, there is evidence that apomeiotic hybrids lacking parthenogenetic capacities are rarely encountered (Nogler 1984a; Kojima et al. 1994). Moreover, in *Tripsacum* species, as in most apomicts, the presence of the gene(s) responsible for apomeiosis generally results in maternal progenies (Nogler 1984a; Asker and Jerling 1992). This may be interpreted in two ways: either the region controlling diplospory contains a major factor with pleiotropic effects on the other essential components of gametophytic apomixis, or it contains several tightly linked factors each controlling one single component. The risk of selecting purely apomeiotic or diplosporous genotypes in derivatives of *T. dactyloides* by using markers co-segregating with diplospory would, therefore, be proportional to the strength of this linkage.

## Perspectives

As already reported by Harlan and de Wet (1977), the pathway of genetic transfer from tetraploid *Tripsacum* to maize goes through (1) maize-*Tripsacum* hybrid backcross generations carrying a decreasing number of *Tripsacum* chromosomes plus the two complete sets of maize chromosomes; and (2) the production of "tripsacoid" maize plants from maize-*Tripsacum* addition lines combining 20 chromosomes from maize and a few from *Tripsacum* (three being the most frequent *Tripsacum* chromosome number).

Such a scheme becomes more complex when the transfer of apomixis is attempted, as a result of the male sterility that drastically affects the first backcross generations (Harlan and de Wet 1977) and of the high numbers of useless individuals derived through apomixis. However, in some rare cases, reduced egg cells are observed (Leblanc et al. 1995), allowing the production of sexual off-types that are the only ones useful for the achievement of the backcross scheme. The determination of the mode of reproduction can be carried out using either cytoembryological analyses in ovules or progeny-tests performed one generation latter (Bashaw 1980). Such procedures, however, are destructive (ovule analyses), time-consumming and cannot be carried out before flowering, making them difficult to apply when large numbers of progenies have to be screened and/or when low female fertility occurs. Marker-based selection helps overcome these limitations, allowing an early identification of apomictic geno-

types.

The markers reported here will simplify the identification of chromosome addition lines carrying the chromosome of interest, and ultimately maize-*Tripsacum* recombinants. In order to attempt the production of apomictic maize, enhancement of pairing between homoeologous segments of the maize and *Tripsacum* genomes will be attempted following a strategy based on B-A translocations in maize (Beckett 1978). Cytological studies of maize-*Tripsacum* hybrids have shown that B-A translocations might be effective tools for chromosome manipulation and the introduction of *Tripsacum* DNA segments into maize (Kindiger and Beckett 1990).

Wild tetraploid plants obviously do not represent an attractive system for gene isolation via map-based gene cloning. However, if the transfer of apomixis into maize is achieved, a number of avenues for the molecular characterization and isolation of the genes of interest, such as map-based cloning or transposon tagging, open up. Eventually, the introduction of apomixis into other crops through genetic engineering might become possible.

## References


Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Asker S, Jerling L (1992) Apomixis in plants. CRC Press, Boca Raton

Bashaw EC (1980) Apomixis and its implication in crop improvement. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. American Society of Agronomy, Madison, Wisconsin, pp 45–63

Beckett JB (1978) B-A translocations in maize. J Hered 69:27–36

Bennetzen JL, Freeling M (1993) Grasses as a single genetic system: genome composition, collinearity and compatibility. Trends Genet 9:259–261

Brown WV, Emery HP (1958) Apomixis in the Gramineae: Panicoideae. Am J Bot 45:253–263

Burr B, Burr F, Thompson KA, Albertson MC, Stubber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Burua UM, Chalmers KJ, Hackett CA, Thomas WTB, Powell W, Waugh R (1993) Identification of RAPD markers linked to a *Rhynchosporium secalis* resistance locus in barley using near-isogenic lines and bulked-segregant analysis. Heredity 71: 177–184

Crane CF, Carman JG (1987) Mechanisms of apomixis in *Elymus rectisetus* from Eastern Australia and New Zealand. Am J Bot 74:477–496

Dumas C, Mogensen HL (1993) Gametes and fertilization: maize as a model system for experimental embryogenesis in flowering plants. The Plant Cell 5:1337–1348

Faure JE, Digonnet C, Dumas C (1994) An in vitro system for adhesion and fusion of maize gametes. Science 263:1598–1600

Galinat WC (1971) The origin of maize. Annu Rev Gen 5:447–478

Gardiner JM, Coe EH, Melia-Hancock S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized $F_2$ population. Genetics 134:917–930

Grivet L, D'Hont A, Dufour P, Hamon P, Roques D, Glazmann JC (1994) Comparative mapping of sugarcane with other species within the Andropogoneae tribe. Heredity 73:500–508

Harlan JR, de Wet JMJ (1977) Pathways of genetic transfer from *Tripsacum* to *Zea mays*. Proc Natl Acad Sci USA 74:3494–3497

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Hoisington D, Khairallah M, González-de-León D (1994) Laboratory protocols, 2nd edn. CIMMYT Applied Molecular Genetics Laboratory. CIMMYT, México D.F

Hulbert SH, Richter TE, Axtell JD, Bennentzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc Natl Acad Sci USA 87: 4251–4255

Jefferson RA (1994) Apomixis: a social revolution for agriculture? The Monitor 19:14–16

Kindiger B, Beckett JB (1990) Cytological evidence supporting a procedure for directing and enhancing pairing between maize and *Tripsacum*. Genome 33:495–500

Kojima A, Kozono T, Nagato Y, Hinata K (1994) Non-parthenogenetic plants detected in Chinese chive, a facultative apomict. Breeding Sci 44:143–149

Leblanc O, Peel MD, Carman JG, Savidan Y (1995) Megasporogenesis and megagametogenesis in several *Tripsacum* species (Poaceae). Am J Bot 82 (in press)

Liu CJ, Atkinson MD, Chinoy CN, Devos KM, Gale MD (1992) Nonhomoeologous translocations between group 4, 5, and 7 chromosomes within wheat and rye. Theor Appl Genet 83:305–312

Melake Berhan A, Hulbert SH, Butler LG, Bennetzen JL (1993) Structure and evolution of the genomes of *Sorghum bicolor* and *Zea mays*. Theor Appl Genet 86:598–604

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked-segregant analysis: a rapid method to detect markers in specific genome regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Mogie M (1988) A model for the evolution and control of generative apomixis. Biol J Linn Soc 35:127–153

Mohan M, Nair S, Bentur JS, Rao UP, Bennett J (1994) RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of the gall midge (*Orseolia oryzae*). Theor Appl Genet 87:782–788

Nogler GA (1984a) Gametophytic apomixis. In: Johri BM (ed) Embryology of angiosperms. Springer-Verlag, Berlin

Nogler GA (1984b) Genetics of apospory in apomictic *Ranunculus auricomus*. V. Conclusion. Bot Helv 94:411–422

Ozias-Akins P, Lubbers EL, Hanna WW, McNay JW (1993) Transmission of the apomictic mode of reproduction in *Pennisetum*: co-inheritance of the trait and molecular markers. Theor Appl Genet 85:632–638

Peel MD (1993) Meiocyte callose in aposporic and diplosporic grasses and in hybrids between bread wheat and *Elymus rectisetus*. MS thesis Utah State University

Penner GA, Chong J, Lévesque-Lemay M, Molnar SJ, Fedak G (1993) Identification of a RAPD marker linked to the oat stem-rust gene *Pg3*. Theor Appl Genet 85:702–705

Pineda O, Bonierbale MW, Plaisted RL, Brodie BB, Tanksley SD (1993) Identification of RFLP markers linked to the the *H1* gene conferring the resistance to the potato cyst nematode *Globodera rostochiensis*. Genome 36:152–156

Renner O (1916) Zur Terminologie des pflanzlichen Generationswechsels. Biol Zentralbl 36:337–374

Savidan Y (1982) Nature et hérédité de l'apomixie chez *Panicum maximum* Jacq. PhD thesis Université of Paris XI

Savidan Y, Dujardin M (1992) Apomixie: la prochaine révolution verte? La Recherche 241:326–334

Sherwood RT, Berg CC, Young BA (1994) Inheritance of apospory in buffalograss. Crop Sci 34:1490–1494

Stuber CW, Wendel JF, Goodman MM, Smith JCS (1988) Techniques and scoring procedures for starch-gel electrophoresis of enzymes from maize (*Zea mays* L.). North Carolina State Experiment Station Technical Bulletin 286.

Valle CB do, Miles JW (1994) Melhoramento de gramineas do genero Brachiaria. In: Simposio sobre manejo pastagens, 11. Fundacao de Estudos Agrarios Luiz de Queiroz, Piracicaba,