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TRIPSACUM ANDERSONII IS A NATURAL HYBRID INVOLVING ZEA AND TRIPSACUM: MOLECULAR EVIDENCE[1]

LUTHER E. TALBERT,[2] JOHN F. DOEBLEY,[3]
STEVEN LARSON,[3] AND VICKI L. CHANDLER[4]

[2]Department of Plant and Soil Science, Montana State University, Bozeman, Montana 59717;
[3]Plant Biology Department, University of Minnesota, St. Paul, Minnesota 55108; and
[4]Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

ABSTRACT

Cytogenetic evidence suggests that *Tripsacum andersonii* may be a natural hybrid between *Zea* and *Tripsacum*. In this paper we show sequences that hybridize to the transposable elements *Mu1* and *Spm* are found in *T. andersonii* and all *Zea* species examined. However, no hybridizable sequences are observed in the five other *Tripsacum* species surveyed. These results suggest that *Mu* and *Spm* elements became components of the *Zea* genome after the divergence of *Zea* and *Tripsacum*, and they strongly support the cytological evidence that *T. andersonii* is a *Zea-Tripsacum* hybrid. Examination of nuclear ribosomal genes of *T. andersonii* also supports the hybridization hypothesis and identifies the *Zea* parent as *Zea luxurians*. The *Tripsacum* parent could not be conclusively identified, but the ribosomal gene data suggest that the species of *Tripsacum* section *Fasiculata* most closely resemble *T. andersonii*. Restriction site patterns of two chloroplast DNA sequences indicate that the maternal parent was a species of *Tripsacum*. These results are complemented by morphological evidence regarding the origin of *T. andersonii*.

TRIPSACUM (x = 18) is considered to be the genus most closely related to *Zea* (x = 10). Both are monoecious grasses indigenous to the Western hemisphere. *Zea* includes cultivated maize (*Zea mays* L. ssp. *mays*) and its wild and weedy relatives known as teosintes (Doebley and Iltis, 1980; Iltis and Doebley, 1980). At least 15 species are recognized in *Tripsacum* (deWet, Gray, and Harlan, 1976). Experimental hybrids have been produced between cultivated maize and several *Tripsacum* species (MaGuire, 1964; Reeves and Bockholt, 1964; Mangelsdorf, 1961; deWet and Harlan, 1974). All experimentally produced hybrids have been male sterile.

Cytological evidence suggests that *T. andersonii* J. R. Gray may be a natural *Zea-Tripsacum* hybrid. *T. andersonii* is sterile and contains $2n = 64$ chromosomes (Levings, Timothy, and Hu, 1976). At least ten chromosomes remain unpaired during meiosis, and the remaining 54 chromosomes appear to be three homologous sets of the 18 *Tripsacum* chromosomes (deWet and Harlan, 1979; deWet et al., 1983b). Thus, an attractive hypothesis is that *T. andersonii* is a natural hybrid containing three sets of *Tripsacum* chromosomes and a haploid component of *Zea* chromosomes (deWet and Harlan, 1979; deWet et al., 1983b).

Species of interspecific hybrid origin are common among vascular plants. Often hybridization is accompanied by chromosome doubling (allopolyploidy), which enables the hybrid species to reproduce sexually. Less frequently, the haploid chromosome numbers of the parental species are preserved and the capability to reproduce sexually is lost (Grant, 1981). Such apomictic hybrid species can propagate by vegetative or agamospermous means (Grant, 1981). *Tripsacum andersonii* is of interest because it appears to be one of the few examples of this less frequent mode of hybrid speciation. Moreover, as we will demonstrate, it is of intergeneric rather than interspecific origin.

Molecular (DNA) markers provide a powerful method of tracking introgression and hybridization among plant species. Recent molecular characterization of *Tripsacum* and *Zea* has provided a background for addressing the potential hybrid nature of *Tripsacum andersonii*. In this manuscript, we were initially interested in determining whether *T. andersonii* indeed contains a genome from *Zea*. Two lines of evidence suggest that *Mu* transposable elements may be useful molecular markers for assessing *Zea* hybridization with *Tripsacum*.

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First, approximately 20–40 *Mu* elements may be visualized on Southern blots in all *Zea* species using a probe for the highly conserved *Mu* terminal inverted repeats (Chandler, Rivin, and Walbot, 1986; Talbert and Chandler, 1988; Talbert, Patterson, and Chandler, 1989). Second, the lack of sequences that hybridize to the *Mu* terminal inverted repeat in *Tripsacum dactyloides* suggests that *Mu* elements may have become a component of the *Zea* genome after its evolutionary divergence from *Tripsacum* (Talbert and Chandler, 1988). Results reported here show that *Mu* elements are present in *T. andersonii* and no other *Tripsacum* species, which strongly suggests that *T. andersonii* contains a genome from *Zea.* Analogous results were obtained with the maize transposable element *Spm.* Additionally, ribosomal DNA variation among *Zea* species (Zimmer, Jupe, and Walbot, 1988) allowed us to more precisely delineate the *Zea* progenitor, while restriction site variation between the chloroplast genomes of *Tripsacum* and *Zea* (Doebley, Renfroe, and Blanton, 1987) allowed determination of the maternal parent. Interpretation of molecular results is consistent with morphological considerations.

MATERIALS AND METHODS—*Plant materials*—The genus *Zea* has been divided into two sections containing a total of four species (Doebley and Iltis, 1980). In this study, representatives of Section *Zea* included *Zea mays* subsp. *huehuetenangensis* (Iltis G-120), subsp. *parviglumis* (Puga 11065), subsp. *mexicana* Race Chalco (Doebley 649), and subsp. *mays* (Inbred B37, Race Conico or Race Reventador). Representatives of Section *Luxuriantes* included *Zea luxurians* (Iltis G-5), *Zea diploperennis* (Guzman 777), and *Zea perennis* (Iltis et al. 1050). *Tripsacum* species analyzed included *T. pilosum* (Doebley 467), *T. dactyloides* (Doebley 644), *T. latifolium* (Timothy 79-20), *T. andersonii* (Timothy 68-68), *T. laxum* (Timothy 79-3), *T. maizar* (Timothy 79-29), and *T. peruvianum* (Timothy 66-9). All *Tripsacum* species except *T. dactyloides* were generously provided by D. H. Timothy, North Carolina State University.

Probes—A probe specific for the *Mu* terminal inverted repeat was prepared from the plasmid pDTE1 as described by Chandler et al. (1986). A *Spm*-specific plasmid pEco 1.25 was obtained from R. J. Schmidt, University of California, San Diego. This plasmid is an internal subclone of the *Spm* element cloned from the *opaque-2* locus (Schmidt, Burr, and Burr, 1987). The plasmid clone (pZmr1) of the maize 18S-26S ribosomal repeat was obtained from R. L. Phillips, University of Minnesota. Chloroplast probes (Lambda 9 and 11) were described by Larrinua et al. (1983).

Southern blot hybridizations—Plant leaf DNA was isolated from single plants per accession using either the method of Dellaporta, Wood, and Hicks (1983) or Saghai-Maroof et al. (1984). Southern blot hybridizations were conducted as described by Chandler et al. (1986) or Doebley et al. (1987). All probes were labeled by the random hexamer primer reaction (Feinburg and Vogelstein, 1983).

RESULTS AND DISCUSSION—Our initial interest was to determine whether *Tripsacum andersonii* contains a genome from *Zea.* Previous work suggested that *Mu* elements are found only in the genus *Zea.* Thus, the presence of *Mu* elements in *T. andersonii* would be strong evidence for the existence of a *Zea* genome in this species. A Southern blot with DNA from several *Zea* and *Tripsacum* species hybridized to a probe for the *Mu* terminal inverted repeat is shown in Fig. 1A. *Mu*-hybridizing bands are observed in *Zea mays* subsp. *mays* (lane a), *Zea perennis* (lane b), *Zea diploperennis* (lane c), *T. andersonii* (lane f), and *Zea luxurians* (lane h). Conversely, no hybridizing sequences are observed in *Tripsacum latifolium* (lane d), *Tripsacum peruvianum* (lane e), or *Tripsacum dactyloides* (lane g). No *Mu*-hybridizing sequences were observed in two other *Tripsacum* species, *Tripsacum laxum* and *Tripsacum pilosum* (data not shown). A similar result was obtained with the *Spm* probe, in that hybridizing bands were observed in all *Zea* species and *T. andersonii,* but no *Spm*-hybridizing bands were observed in the other *T. Tripsacum* species (Fig. 1B). This evidence strongly supports the hypothesis that *Mu* and *Spm* elements became components of the *Zea* genome after divergence from *Tripsacum,* and that *T. andersonii* is a hybrid with *Zea.*

We were also interested in determining the *Zea* and *Tripsacum* parents of *Tripsacum andersonii.* Four species and two sections are recognized in *Zea* (Doebley and Iltis, 1980). Using ribosomal DNA polymorphisms as markers, Zimmer et al. (1988) found that the three species within section *Luxuriantes* (*Z. luxurians, Z. diploperennis,* and *Z. perennis*) could be differentiated from *Zea mays,* the only species recognized in section *Zea.* In particular, the three species in section *Luxuriantes* possess an *Eco*RI site that digests the 9.6-kb ribosomal

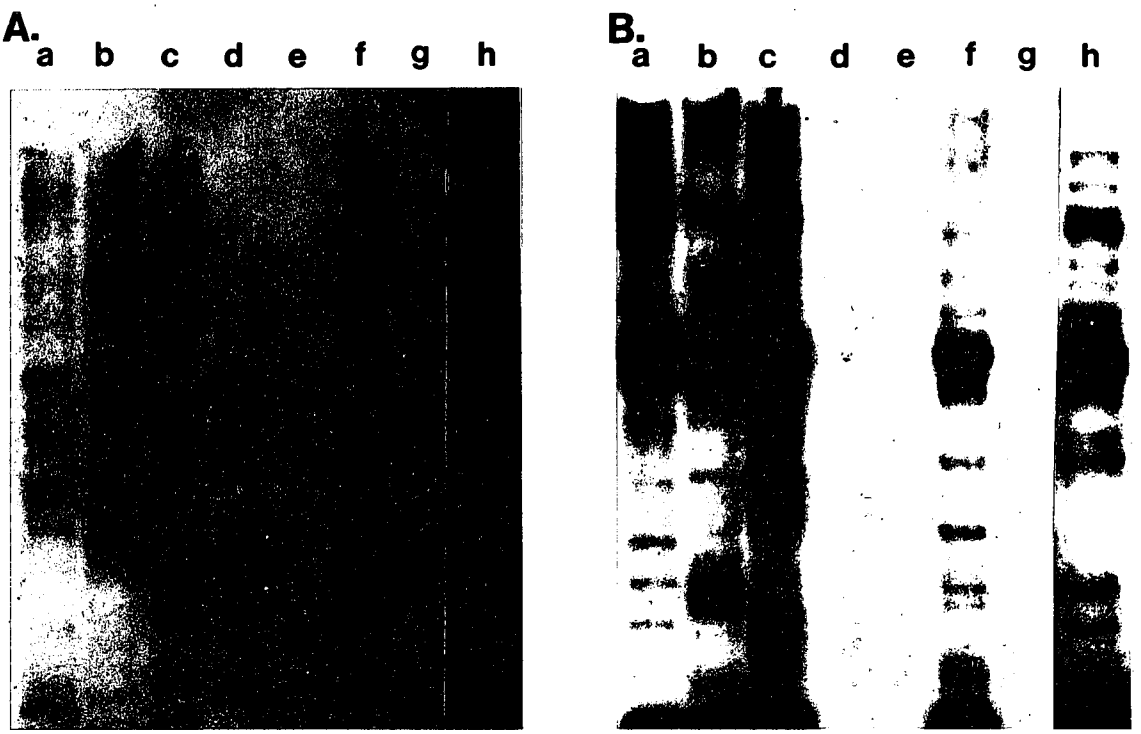

Fig. 1. Presence of *Mu* and *Spm* elements in *Zea* and *Tripsacum* species. Approximately 6 μg of DNA was digested with *Eco*RI and *Hin*DIII for each of the species. Lane a: *Zea mays* subsp. *mays*; b: *Zea perennis*; c: *Zea diploperennis*; d: *Tripsacum latifolium*; e: *Tripsacum peruvianum*; f: *Tripsacum andersonii*; g: *Tripsacum dactyloides*; h: *Zea luxurians*. Panel A. Blot hybridized to a probe for the *Mu* terminal inverted repeat. Panel B. Blot hybridized to a probe specific for *Spm*. For both probes blots were washed at 58 C in 0.1× sodium dodecyl sulfate.

repeats into 6.5 and 2.8–3.1-kb fragments. Further, the 2.8-kb fragment is specific to *Z. luxurians*, and the 3.1-kb fragment is specific to *Z. perennis* and *Z. diploperennis*. This *Eco*RI site was absent in representatives of section *Zea* (*Zea mays* subsp. *mays* and *Zea mays* subsp. *mexicana*) and the five *Tripsacum* species examined (Zimmer et al., 1988). Thus, the presence of these fragments in *T. andersonii* could be used to identify the *Zea* parent of this

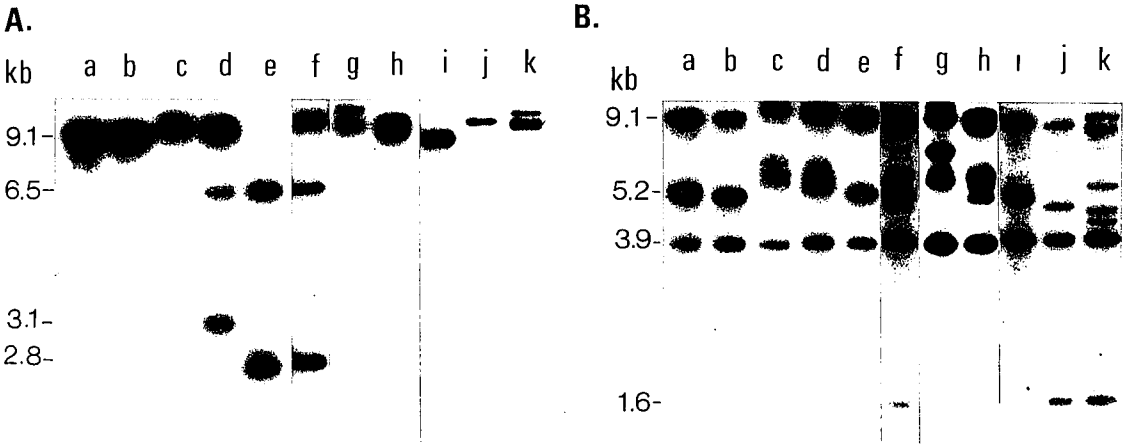

Fig. 2. Autoradiographs of Southern blots probed with the maize 18S-26S ribosomal repeat clone (pZmr1). Lane a: *Zea mays* subsp. *mays*; b: *Z. mays* subsp. *mexicana*; c: *Z. perennis*; d: *Z. diploperennis*; e: *Z. luxurians*; f: *T. andersonii*; g: *T. latifolium*; h: *T. peruvianum*; i: *T. dactyloides*; j: *T. laxum*; k: *T. maizar*. Panel A. *Eco*RI. Panel B. *Bam*HI. Fragment sizes are indicated in kilobases (kb).

sterile, intergeneric hybrid. Figure 2A shows a Southern blot with *Eco*RI-digested DNA from several *Tripsacum* and *Zea* species. Hybridizing fragments of 6.5 and 2.8 kb are observed in *Zea luxurians* (lane e) and *T. andersonii* (lane f). These fragments are not in the other *Tripsacums* (lanes g–k). *Zea perennis* (lane c) and *Z. diploperennis* (lane d) possess the 6.5-kb fragment but have a 3.1-kb fragment in place of the 2.8-kb one. (In our sample of *Z. perennis,* the 6.5- and 3.1-kb fragments are seen only with longer exposures of the x-ray film.) These data indicate that the *Zea* parent of *T. andersonii* was *Z. luxurians.*

Some evidence regarding the *Tripsacum* parent was obtained from a *Bam*HI digest of the DNAs followed by probing with the ribosomal gene probe (Fig. 2B). Of particular interest here is the presence of a 1.6-kb fragment in *T. andersonii* (lane f), *T. laxum* (lane j), and *T. maizar* (lane k). The latter two species both belong to section *Fasiculata* of *Tripsacum,* suggesting that a species of this section may have been the *Tripsacum* parent. *T. andersonii* has previously been placed in section *Tripsacum* (deWet et al., 1976), a placement that the molecular evidence does not support.

Differences in maternally inherited chloroplast DNA from *Zea* and *Tripsacum* enabled us to establish the maternal parent of *T. andersonii.* Previous work (Doebley et al., 1987) has shown a set of restriction site mutations that distinguish all *Zea* species, including *Zea luxurians,* from *Tripsacum.* We assayed *T. andersonii* for these sites in order to determine its maternal parent. Southern blots with *Eco*RI- and *Bam*HI-digested total cellular DNA were probed with two cloned portions (lambda 9 and 11) of the maize chloroplast genome (data not shown). By this, we were able to determine the presence of three restriction site mutations (No. 9, 15, and 16) that distinguish the *Zea* and *Tripsacum* chloroplast genomes (Doebley et al., 1987). These analyses demonstrated that *T. andersonii* possesses a *Tripsacum* chloroplast genome, and thus indicate that its maternal parent was a *Tripsacum.*

In conclusion, our data confirm the cytological evidence that *Tripsacum andersonii* is a natural hybrid of *Zea* and *Tripsacum.* However, our data differ with the interpretation that *Z. mays* and *T. latifolium* were the parental species (deWet et al., 1983b). Ribosomal gene restriction site variation provides evidence that the *Zea* parent was *Z. luxurians* and not *Z. mays.* This result concurs with the morphological observation of one of us (JD) that both *Z. luxurians* and *T. andersonii* have more highly nerved outer glumes than other members of these genera. Further, the female fruitcases of *T. andersonii* do not show any of the morphological features (e.g., paired spikelets) often found in *Tripsacum* hybrids with domesticate maize. Our data also indicate that the *Tripsacum* parent was a member of section *Fasiculata.* It is noteworthy that several members of this section including *T. laxum, T. maizar,* and *T. pilosum* grow in the same region of Guatemala as *Z. luxurians* (deWet, Brink, and Cohen, 1983a; Doebley, 1983).

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