

Genetic relations among *Tripsacum* species revealed by genomic variation

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Introduction

We studied genomic variation among several *Tripsacum* spp. and teosinte species in collaboration with Floragenex Inc., Portland, Oregon, USA. Materials were 18 *Tripsacum* clones from Mexico and South America, seven teosinte race populations, and one tropical maize inbred (tables 1, 3, and S1). A preliminary analysis revealed interesting results for relationships between *Zea* and *Tripsacum*.

Sequencing and SNP (polymorphism) discovery

Paired-end Illumina / Solexa sequence reads of the material were anchored to the *Tripsacum* clone sample 12 (table S1) reference assembly, using custom short-read software with a Needleman-Wunsch alignment algorithm. Specified alignment thresholds allowed approximately three base pair changes between the ~50 bp Illumina / Solexa read and the reference (>90 percent identity). Small gapped alignments (short indels) spanning one or two base pairs were also permissible.

A total of 25,878 contigs were constructed, with 5,705,986 base pairs. Contig sequence length ranged from 100 to 625 bp, with an average of 220.5 bp. Using the *Tripsacum* clone 12 as the reference, the number of contigs with at least one polymorphism was 11558. Within the 11558 contigs, 7756 were aligned onto the B73 genome, where 45449 polymorphic loci were anchored.

In total, 72413 polymorphic loci were identified. Of these, 1036 were high-quality polymorphic loci identified across all 26 lines; 10 of which were InDel genomic variation and 1026 were single nucleotide polymorphisms (SNPs).

Clustering analysis of *Tripsacum*, Teosinte, and maize

Information on all the material analyzed in this study is shown in tables 1, 2, and 3.

Table 1. *Tripsacum* clones sampled for this study at CIMMYT Tlaltizapán station, 2009.

Clonal sample ID	Collection No.	Population No.	Species classification	chromosome number	Possible ploidy	Country of origin	Collection site and province or state	Collection site altitude (m), latitude, longitude
01	T.1069	95	TMN (<i>T. manisuroides</i> de Wet and Harlan)	36	diploid	Mexico	Mirador, Los Chiapas, Chiapas	1200, 16:37; -91:85
02	T. 5243	613	TCD (<i>T.cundinamarce</i> de Wet and Timothy)	36	diploid	Colombia	Viota-Tamarindo, Cundinamarca	1095, 4:42; -74:52
03	T. 5222	603	TMR (<i>T.dactyloides</i> var. <i>meridionale</i> de Wet and Timothy)	36	diploid	Venezuela	El Caimito-5km from Libertad, Tachira	1400, 7:58; -72:32
04	T.7003	1	TZP (<i>T. zopilotense</i> Hernandez and Randolph)	36	diploid	Mexico	Cañon de Zopilote, Guerrero	354, 18:35; -101:70
05	T. 5087	554	TMR (<i>T.dactyloides</i> var. <i>meridionale</i> de Wet and Timothy)	36	diploid	Colombia	Sota, 7km Carr. a Sota, Boyaca	1000, 6:32; -72:70
06	T.69	18	TLT (<i>T.latifolium</i> Hitchc.)	54	triploid	Mexico	Pluma Hidalgo, Oaxaca	1100, 15:92; -96:42
07	T.7146	4	TMZ (<i>T. maizar</i> Hernandes and Randolph)	54	triploid	Mexico	San Jose II, Nayarit	30, 22.47; -105:30
08	T.552	6	TBV (<i>T. bravum</i> Gray)	72	tetraploid	Mexico	Rancho Nuevo, 87.7 km from Iguala, Guerrero	780, 18:17; -101:40
09	T.569	9	TDH (<i>T. dactyloides</i> var. <i>hispidum</i> (Hitchc.) de Wet and Harlan)	72	tetraploid	Mexico	Zacazonapan, 2 km N. of Zacazonapan, Mexico	1450, 19:07; -100:20
10	T.139	24	TDM (<i>T.dactyloides</i> var. <i>mexicanum</i> de Wet and Harlan)	72	tetraploid	Mexico	Aguascaliente, 13km S. of Acatlán de Juarez, Jalisco	1420, 19:98; -104:00
11	T.210	31	TIT (<i>T. intermedium</i> de Wet and Harlan)	72	tetraploid	Mexico	Ojo de Agua, 1km W. of Ojo de Agua, Colima	170, 19:32; -103:80
12	T.1070	96	TJL (<i>T.Jalapense</i> de Wet et Brink)	72	tetraploid	Mexico	La Coyota, km 14.9 Carr. Sumidero, Chiapas	1150,
13	T.7221	1	TPL (<i>T.pilosum</i> Scribner and Merrill)	72	tetraploid	Mexico	Jilotlán, Jalisco	740, 19:22; -103:01
14	T.1610	39	TDM (<i>T.dactyloides</i> var. <i>mexicanum</i> de Wet and Harlan)	72	tetraploid	Mexico	La Toma, 3 km W. of Tequila, Jalisco	1200, 20:90; -103.80
15	T.2131	142	LC (<i>T.lanceolatum</i> Ruprecht ex Fournir)	72	tetraploid	Mexico	Rio Chico, Carr. Mazatlán, Durango	2100, 23:93; -104.80
16	T.2368	153	TDD (<i>T. dactyloides</i> (L.) L.)	72	tetraploid	Mexico	Lamasinta, Carr. Chilapa-Tlapa, Guerrero	1500, 17:60; -99:17
17	T.5081	550	TPR (<i>T.peruvianum</i> de Wet and Timothy)	90	pentaploid	Peru	Tingode Saposoa, San Martin	299, -7:12; -76:62
18	T. 5023	522	TAD (<i>T.andersonii</i> Gray)	64	hybrid (64)	Brazil	Campinas Institute of Agronomy, Sao Paulo	661, -22:88; -46:07

Table 2. Plant traits measured for the *Tripsacum* clones analyzed in this study and for some additional clones at CIMMYT Tlaltizapán station.

Clonal Sample ID	Collection No.	Popultion No.	Species classification	Plant height (m)	Leaf length (cm)	Leaf width (cm)	Culm width (cm)	Culm thickness (cm)
<i>Tripsacum</i> clones used for phylogenetic study in A1 plot at Tlaltizapán, Morelos. Mexico								
01	T.1069	95	TMN (<i>T. manisuroides</i> de Wet and Harlan)	3.28	152	3.3	1.5	1.35
02	T. 5243	613	TCD (<i>T.cundinamarce</i> de Wet and Timothy)	3.15	158	4.2	2.15	1.65
03	T. 5222	603	TMR (<i>T.dactyloides var.meridionale</i> de Wet and Timothy)					
04	T.7003	1	TZP (<i>T. zopilotense</i> Hernandez and Randolph)	2.39	144	1.6	0.6	0.4
05	T. 5087	554	TMR (<i>T.dactyloides var.meridionale</i> de Wet and Timothy)	2.5	127	2	1.2	1
06	T.69	18	TLT (<i>T.latifolium</i> Hitchc.)	1.8	135	4.1	2.3	2
07	T.7146	4	TMZ (<i>T. maizar</i> Hernandes and Randolph)	2.07	128	6	2.3	2.15
08	T.552	6	TBV (<i>T. bravum</i> Gray)	1.68	114	1.9	1.4	1.15
09	T.569	9	TDH (<i>T. dactyloides var. hispidum</i> (Hitchc.) de Wet and Harlan)	1.15	122	1.43	0.7	0.55
10	T.139	24	TDM (<i>T.dactyloides var.mexicanum</i> de Wet and Harlan)	3.2	177	3.13	1.7	1.25
11	T.210	31	TIT (<i>T. intermedium</i> de Wet and Harlan)	3.28	179	3.66	0.95	0.8
12	T.1070	96	TJL (<i>T.Jalapense</i> de Wet et Brink)	3.24	178	3.6	1.25	1.1
13	T.7221	1	TPL (<i>T.pilosum</i> Scribner and Merrill)	3.53	223	4.1	2.2	1.7
14	T.1610	39	TDM (<i>T.dactyloides var.mexicanum</i> de Wet and Harlan)	2.77	155	3.67	1.8	1.5
15	T.2131	142	LC (<i>T.lanceolatum</i> Ruprecht ex Fournir)	1.64	129	1.65	0.7	0.5
16	T.2368	153	TDD (<i>T. dactyloides</i> (L.) L.)	2.5	150	0.76	0.65	0.45
17	T.5081	550	TPR (<i>T.peruvianum</i> de Wet and Timothy)	2.73	140	4.1	1.5	1.1
18	T. 5023	522	TAD (<i>T.andersonii</i> Gray)	2.08	163	6.73	2.5	2.1
Additional <i>Tripsacum</i> clones measured in A0 plot at Tlaltizapán, Morelos, Mexico								
A01	TZP-DEC- 0506		TZP (<i>T. zopilotense</i> Hernandez and Randolph)	2.18	142	0.9	0.35	0.3
A02	T.5085	553	TPR (<i>T.peruvianum</i> de Wet and Timothy)	2.14	129	2.96	0.7	0.55
A03	TLX-DEC-05-72		TLX(<i>T. laxum</i> Nash)	4.4	144	5.1	2.15	1.65
A04	TLT-DEC-05-70		TLT (<i>T.latifolium</i> Hitchc.)	2.95	203	3.46	1.85	1.05

Table 3. Maize and teosinte samples included in this study.

Sample ID	CIMMYT Bank ID	Race name	Population	Collection site or seed origin
Maize line	16417	Tuxpeño pool	CML 247	G24F119*G24F54-6-4-1-1-BB,CIMMYT
Teosinte 01	8765	Balsas	K67-22	Tinganbato, Michoacán, Mexico
Teosinte 02	8768	Chalco	K68-1	Milpa Alta, México, Mexico
Teosinte 03	9478	Guatemala		Chiquimula, Guatemala
Teosinte 04	9479	Huehuetenango		Jacaltanango, Huehuetenango, Guatemala
Teosinte 05	10003	Diploperennis	San Miguel	Cautitlán, Jalisco, Mexico
Teosinte 06	11367	Central Plateau	K69-5	Chacandiro, Michoacán,
Teosinte 07	11403	Balsas	W.S.92-12	Ixcapzalco, Guerrero, Mexico

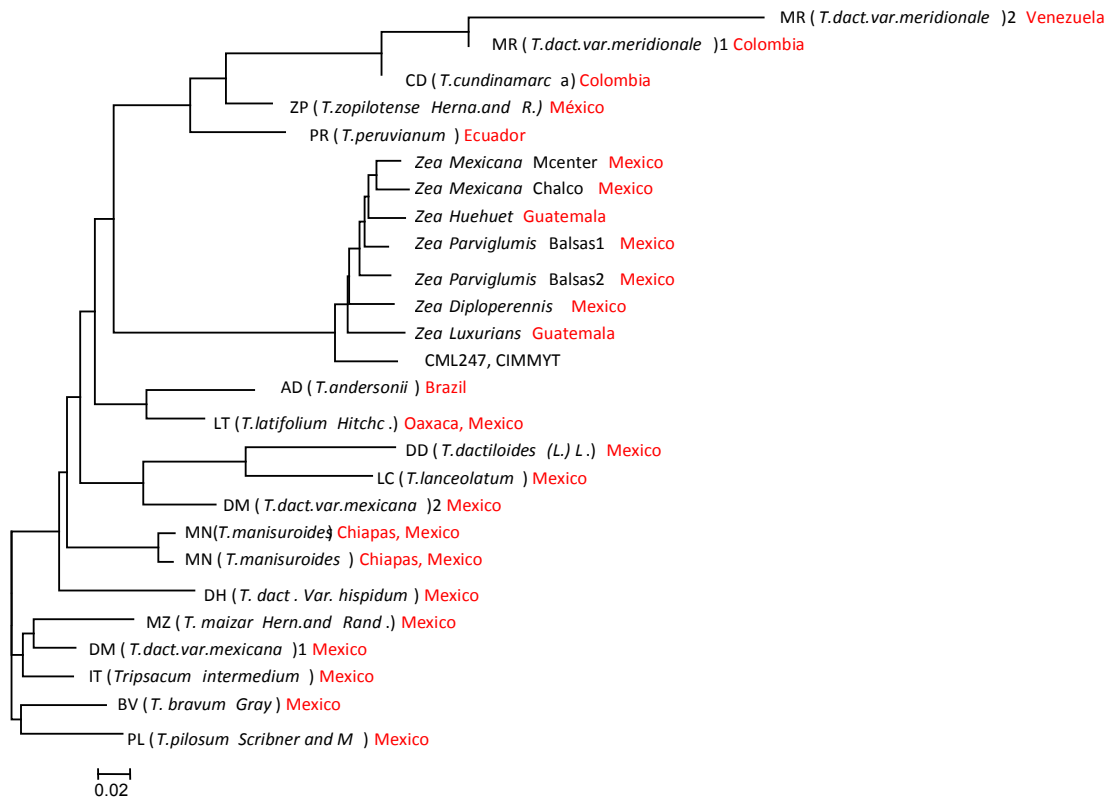
**Figure 1.** Neighbor-joining tree of *Tripsacum*, teosinte, and maize, which was constructed based on the Rogers distance, calculated using 1036 SNPs.

Figure 1 shows that among the *Tripsacum* species studied, those from South America (i.e., *T. peruvianum*, *T. cundinamarca*, and *T. dactyloides*) were genetically closest to *Zea* (teosinte and maize), though *T. zopilotense* (Mexico) had similar results. The next closest species to *Zea* were *T. latifolium* and *T. andersonii*.

The teosinte species were clustered as shown in Figure 1. *T. zopilotense* reportedly crosses well with maize (Savidan et al. 1993) and with *T. australe* (Raymundo Q. C. 1993). *T. dactyloides* is

also known to cross well with maize. Unfortunately we did not have *T. australe* and *T. jalapense* in our study.

From the current relationship among the taxa of *Tripsacum*, teosinte, and maize, we recommended that inter-specific gene pools of *Tripsacum* species and *Tripsacum* X maize gene pools are made to exploit useful genes and allelic diversity (Li et al. 1999; Dewald and Kindiger 1998) that may exist in *Tripsacum*.

It took many days to curate a *Tripsacum* garden in Tlaltizapán station. The passport data of exiting clones are updated. Some clones from the South American highlands of South America suffered from high temperatures at the station.

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Table S1. Number of Illumina Sequence Reads obtained in *Tripsacum* and *Zea* accessions in this study

Accession (DNA sample ID)	Number of reads
maize line-CML247	1,527,024
Teosinte01	179,916
Teosinte02	208,823
Teosinte03	249,833
Teosinte04	330,936
Teosinte05	269,964
Teosinte06	350,930
Teosinte07	806,423
Tripsacum01	2,052,542
Tripsacum02	639,275
Tripsacum03	480,868
Tripsacum04	484,349
Tripsacum05	609,107
Tripsacum06	457,786
Tripsacum07	692,378
Tripsacum08	573,691
Tripsacum09	844,068
Tripsacum10	858,477
Tripsacum11	729,792
Tripsacum12	4,183,737
Tripsacum13	2,367,395
Tripsacum14	3,715,711
Tripsacum15	82,867
Tripsacum16	230,727
Tripsacum17	96,609
Tripsacum18	158,771
Total	23,181,999