breeding and negligible non-random mating within each population. Significant variation in the F_{ST} values was found among accessions, with a mean of 0.38, indicating high genetic differentiation between accessions. AMOVA using Arlequin v2.0 revealed that 60% of the genetic variation in these accessions was within the individuals, while 37% of the variation was among populations within a group.

More extensive efforts are in progress at the Maize Genetics Unit, IARI, New Delhi, with regard to phenotypic and molecular characterization of landraces in India. The goals are to identify 'core collections' with potential utility in breeding programmes, and in mining favourable alleles towards influencing productivity and other target traits.

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Evolutionary divergence of the genes *dek1* and *Agpsem* (*agp1*) of *Tripsacum* and *Zea mays*

--Mglinets, A; Sokolov, V; Blakey, A; Tarakanova, T

Eastern gamagrass (*Tripsacum dactyloides* L.), a perennial cereal, is a distant relative of maize and is found widely throughout South and Central America, and southern and eastern areas of North America. Several cytotypes are encountered in nature, diploid *(2n=36)*, triploid *(2n=3x=54)*, tetraploid *(2n=4x=72)* and hexaploid *(2n=6x=108)* forms. Diploids follow a strictly sexual mode of reproduction, whereas polyploids are apomictic. Hybridisation among these species may occur spontaneously. Thus, *Tripsacum andersonii* J.R. Gray, a *Zea* (maize) x *Tripsacum* hybrid with 54 chromosomes which clonally reproduces through budding, has been found in nature and is considered to be a spontaneous hybrid of the two species *Tripsacum dactyloides* and *Zea mays*.

Modern maize x *Tripsacum* hybridisations are also used in plant breeding, primarily for introgression of various agronomic traits. These hybridisations have made it possible to improve maize resistance to high temperatures, to obtain valuable forms of polyunsaturated fatty acids, and to produce apomictic maize x *Tripsacum* hybrids. The use of both molecular mapping and molecular-cytological methods (FISH) has demonstrated that there is a high degree of homology between maize and *Tripsacum* genomes. Molecular probes derived from maize genes can be used for marking, or tagging, *Tripsacum* chromosomes. However, the precise level of genome homology has yet to be determined.

The goal of this research was to determine the primary sequence structure of some *Tripsacum dactyloides* L. genes and to compare them to candidate orthologous maize genes. A *Tripsacum dactyloides* L. specimen, 4N=72, was obtained from the Tashkent Botanic Garden and used as a representative of the species. This particular specimen is of historic significance in that it originated from the materials of N.I. Vavilov's expedition. In addition, this plant accession has also been used to produce apomictic maize x *Tripsacum* hybrids.

Total DNA extraction was carried out from young leaves using cetyltrimethylammonium bromide (CTAB). The DNA was then quantitated and used in polymerase chain reaction (PCR) amplification of specific fragments. The primer pair used for fragment amplification of gene *dek1* was dek1-F (5'-GGGTGCTTTAACTTCAGTTGCA -3') and dek1-R (5'-GCCANGTTCAAATCCAATAGCTG - 3'). For fragment amplification of gene *Agpsem,* the primers Agp-F (5'- GA-TATCCCNGTCAGCAACTG T - 3') and Agp-R (5' - TTTTGGTANTCCATACGGTAC - 3') were used. PCR was carried out in 20 μl of reactions using BIOTAQ™ Red DNA Polymerase by Bioline Enterprise and 10 ng of the total DNA. Amplification cycle reactions were as follows: initial denaturation — 95^{0}C, 3 min.; amplification for 35 cycles: 94^{0}C, 30 sec., annealing — 56^{0}C, 30 sec., extension — 72^{0}C, 60 sec.; final extension — 5 min. Amplification products were visualized in a 1% agarose gel. Total maize DNA extracted from accession C435 (VIR Collection, St.-Petersburg) was used as control. Determination of initial PCR product sequences was carried out in the Inter-Institutional Centre for DNA Sequencing, SB RAS, Novosibirsk using a ABI PRISM® BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit. The same primers were used for the sequencing and amplification reactions. Only part of the initial PCR product sequences were used in the comparative analysis as determined using direct and reverse primers. The maize gene sequences used for comparison were obtained from GenBank (http://www.ncbi.nlm.nih.gov).

Using the primers dek1-F and dek1-R, the expected PCR product size was 886 nucleotides. The fragment of the putative *Tripsacum dek1* gene was determined to be 797 bases long. Direct comparison of initial sequences of the PCR-generated putative *Tripsacum dek1* gene fragment and that of maize demonstrated very high homology, i.e., 99%, and only 1% of differences, for 6 one-nt replacements and 1 three-nt deletion/insertion (Fig. 1). Three one-nt replacements were localised in an exon region, the other three were found in the intron region of maize gene *dek1*. The *Tripsacum* three-nt deletion was found in the gene exon relative to maize.

			48				264			612		
		27			161			468			739	
T. dactyloides	(1) ..	C ..	T ..	..	A ..	..	G ..	..	A ..	T ..	---	..
Zea mays	(5066) ..	A ..	C ..	..	G ..	..	C ..	..	C ..	C ..	ACT	..

Figure 1. Comparison of nucleotide sequences for the *Zea mays dek1* gene (AY061804) and the putative PCR-generated *dek1* gene of *Tripsacum*. Exons of the *Zea mays dek1* gene are shown in grey.

The maize gene *dek1* (*defective kernel1*) is approximately 24k nucleotides long and plays a very important role through its participation in the structural maintenance and functioning of Ca^{2+} ion trans-membrane transport channels. For the maize *dek1* gene, not only is the whole primary structure known, but also intron and exon positions, as well as the structure of its protein product. Therefore, open coding frames may be determined on the primary sequence of the putative *Tripsacum dek1* gene fragment in the areas corresponding to comparative exons of the maize *dek1* gene, and thus it is possible to determine if revealed comparative sequence differences lead to changes at the level of the amino acid sequence. As seen in Fig. 2, the difference in position 161 between maize and *Tripsacum* leads to amino acid replacement. Replacements at nt positions 468 and 612 do not lead to differences in amino acids, i.e., they are synonymous. Absence of an

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                                                   309
T. dactyloides            DWNLGLCSFRFELLKSRMIVLFVAGTSRAFLISFGVHYW

Zea mays        (277)     DWNLGLCSFRFELLKSRMIVLFVAGTSRAFLVSFGVHYW    (316)

T. dactyloides            YLGHCISYAFVASVLLSAAVSSWLSISNPSVARIDALRSTVIKLREGFRRKGQNSSSNSS

Zea mays        (316)     YLGHCISYAFVASVLLSAAVSSWLSISNPSVARIDALRSTVIKLREGFRRKGQNSSSNSS   (376)

T. dactyloides            EGCGSSVKRSSGSVEAGQNGNATDSMYRSNSQSDGVNWSSIPFDRSNSCQEGRSSDKNID

Zea mays        (376)     EGCGSSVKRSSGSVEAGQNGNATDSMYRSNSQSDGVNWSSIPFDRSNSCQEGRSSDKNID   (436)

                                                     380
T. dactyloides            SARASLAHRSNSCLSAVQDSETAVVSVDRHGDP-TSLVCSSSGLESHGCEPSGS

Zea mays        (436)     SARASLAHRSNSCLSAVQDSETAVVSVDRHGDPTTSLVCSSSGLESHGCEPSGS
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Figure 2. Alignment of the amino acid protein sequences coded by the *Zea mays dek1* gene (AAL38188) and the putative PCR-generated *dek1* gene of *Tripsacum*. Positions in amino acid sequence are presented for *Zea mays*.

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                            39        53             149       202         221        271         285       291
                       30        48             146       183        206          2643       278         288       297
T. dactyloides   (1)   .. T .. T .. A .. AG .. .. - .. C .. A .. C .. G A .. G .. T .. G .. CT .. G .. T .. C .. C ..   (308)

Zea mays    (171646)   .. C .. C .. C .. GA .. .. T .. T .. G .. T .. A G .. T .. A .. A .. -- .. A .. C .. T .. A ..   (171953)

                        308       328       335       349                               403          407       456
                            315       331       348                                 396         405       441
T. dactyloides (308)    A .. A .. A .. G .. A .. - ..   ----------------    .. G .. ----  .. C .. C .. A .. --          (456)

Zea mays    (171953)    G .. T .. C .. T .. G .. T .. TTCCTTTTTTTTTTCT      .. A .. GTAG  .. A .. A .. G .. CT          (172124)

                                                  462        501        512                    521         572
                         459                            483        507        516                     552
T. dactyloides (456)   ..    ---------------------    .. G .. A .. A .. A .. T ..  -------   .. A .. T .. A             (573)

Zea mays    (172124)   ..    GATAATCTAATTAAACCAGTG    .. A .. - .. T .. G .. C ..  GTCCTGA   .. G .. C .. G             (172268)

                                 631         639       666       681       689         720             774
                         618         633        651        674       687          717            744
T. dactyloides (573)     .. T .. T .. CT .. C .. T .. A .. C .. C .. C .. C .. T .. A .. .. A .. C ..                 (776)

Zea mays    (172268)     .. A .. C .. TC .. A .. C .. C .. T .. T .. A .. T .. C .. T .. .. G .. T ..                 (172471)
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Figure 3. Comparison of nucleotide sequences for the *Zea mays Agpsemzm* gene and the putative PCR-generated *Agpsem* gene of *Tripsacum*. Exons of the *Zea mays Agpsemzm* gene are shown in grey.

ACT triplet in position 739 of *T. dactyloides* leads to the absence of a threonine amino acid in the putative protein coded by the gene studied compared to the known protein product of the maize gene.

Gene *Agpsemzm* (*agp1*, AGP, small, embryo, *Zea mays*) encodes the small subunit of embryonic ADP-glucosophosphorylase in maize. Comparison of sequence AY032604 (mRNA sequence) and the sequenced maize genome showed that this gene is located on chromosome 2 (BAC_clones: AC177860.4) position 170446 to 181045 (BAC_clones: AC177860.4-Contig127 : 9462 : 20061 : 1). Thus, the total length of the gene coding area is more than 10k bases.

The expected PCR product size was 933 nucleotide pairs with genomic maize DNA and primers Agp-F and Agp-R. Comparison of amplification products obtained using these oligonucleotides and genomic maize and *Tripsacum* DNAs revealed one PCR product with a close molecular weight in each case. The PCR product obtained from genomic *Tripsacum* DNA was determined to be 775 bases long. Bioinformatic analysis of the sequence revealed that it was homologous to genes coding the small subunit of ADP-glucosophosphorylase in different plant species. It manifests the highest homology with the small subunit of embryonic ADP-glucosophosphorylase (AY032604), indicated as *Agpsemzm*. Therefore, one can hypothesize that we have managed to determine the primary sequence of the putative gene fragment encoding the small subunit of embryonic ADP-glucosophosphorylase in *Tripsacum dactyloides* which, in analogy with maize, is indicated as *Agpsem* (AGP, small, embryo).

Comparison of the sequence obtained with the *Agpsemzm* gene showed that the maize sequence is 824 bases long (Fig. 3). Almost 13% of differences are conditioned by one- and two-nt replacements, and deletions/insertions of different lengths (two, four, seven and twenty-one nt long) (Fig. 3).

As intron and exon intra-genic regions have not been determined for the gene *Agpsemzm*, thus it may be possible to determine the open coding reading frames, and to see if nucleotide replacements lead to changes in amino acids in the putative *Tripsacum Agpsem* gene in the comparative regions of maize. Further analysis revealed that all single-nt replacements within the putative *Tripsacum Agpsem* gene fragment corresponding to the second and third maize exons are synonymic (Fig. 4). The two-nt replacement “GA” in maize for that of “AG” in *Tripsacum*

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                                        149
T. dactyloides              LTQFNSASLNRHLSRAYENNIAGYKNEGFVEVLAAQQSPENPNWFQ

Zea mays         (132)      LTQFNSASLNRHLSRAYGNNIAGYKNEGFVEVLAAQQSPENPNWFQ      (177)

T. dactyloides              QGTADAVRQYMWLFEEH

Zea mays         (177)      QGTADAVRQYMWLFEEH        (193)
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Figure 4. Protein amino acid sequences coded by the *Zea mays Agpsemzm* gene (NP_001105178) and the putative PCR-generated *Agpsem* gene of *Tripsacum*. Amino acid positions are indicated for *Zea mays*.

leads to the replacement of a glycine (maize) with a glutamine acid (*Tripsacum*).

The major differences between the nucleotide sequences of the maize *Agpsemzm* gene and the putative *Tripsacum Agpsem* gene were observed in the intron regions, whereas the coding sequence remains conserved. However, by comparison, the sequence differences found in both introns and exons between the maize *dek1* gene and putative *Tripsacum dek1* gene were not as varied. This fact is both very interesting and points to the need for further research.

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Studies on secondary traits of maize inbreds, hybrids and composites across environments

--Devi, P; Singh, NK

Yield stability, as a selection trait in plant breeding programmes as well as in evaluation trials, is constantly gaining importance over yield capacity. This is especially important where environmental conditions vary considerably.

The present study was undertaken during the monsoon season of 2007 in three environments: normal conditions, low nitrogen and irrigated conditions, and low nitrogen and rain (non-irrigated) conditions. Five inbred lines, 10 single crosses and two standard checks, namely Surya (composite) and Nath Samrat 1133 (hybrid), were used as experimental materials with the objective of identifying stable genotypes for the secondary traits, anthesis-silking interval (ASI) and days to 75% ear leaf senescence. The evaluation trials were conducted in each environment in a randomized complete block design with three replications at the Crop Research Centre of the G. B. Pant University of Agriculture and Technology, Pantnagar. The experimental unit was a one row plot 5 m long and 75 cm apart, forming a plot size of 3.75 m^2 and a plant-to-plant distance of 25 cm. The stability of the characters for each genotype was calculated by regressing the mean values of individual genotypes on environmental index and by calculating the deviations of the regression coefficients from unity as suggested by Eberhart and Russell (Crop Sci. 6:36-40, 1966).

The pooled analysis of variance revealed significant differences among genotypes, environments and their interaction for both traits. Inbred lines P_2 and P_3 were found to be the most stable and desirable, whereas single crosses P_1xP_2, P_2xP_3, P_3xP_5 and standard check Surya were identified as ideal in terms of grain yield potential and stability parameters for both the ASI and days to 75% ear leaf senescence (Table).

Table. Stability parameters for anthesis-silking interval (ASI) and days to 75% ear leaf senescence.

Genotypes	Grain yield (kg/ha)	ASI (days)			Days to 75% ear leaf senescence		
	$\overline{X}$ i	$\overline{X}$ i	bi	S^2d_i	$\overline{X}$ i	bi	S^2d_i
Parents							
Pop 31 (P_1)	769.73	4.09	0.877	0.033	80.50	2.539	11.005**
Pop 446 (P_2)	889.29	3.92	0.744	-0.001	78.83	1.735	-0.405
YHP-A (P_3)	1009.72	3.50	0.942	-0.043	80.33	1.312	0.378
Pop 445 (P_4)	737.82	2.67	-0.253**	-0.054	77.00	-0.423*	-0.251
YHP-B (P_5)	858.56	3.17	0.616	2.248**	78.83	-0.375**	-0.546
Crosses							
P_1 x P_2	2184.55	2.50	0.471**	-0.070	80.50	2.253	0.367
P_1 x P_3	1258.50	4.92	1.397	0.140	78.83	3.851	5.318**
P_1 x P_4	1672.48	4.83	1.196**	-0.077	79.83	-0.375**	-0.546
P_1 x P_5	1493.96	4.44	1.341*	-0.040	80.33	0.047*	-0.408
P_2 x P_3	2043.22	4.83	2.429**	0.038	79.94	0.017	0.778
P_2 x P_4	1590.45	3.76	0.418	0.392	79.00	1.126	-0.337
P_2 x P_5	1721.28	2.33	0.725**	-0.074	79.50	3.095*	0.384
P_3 x P_4	1703.05	4.11	1.849**	-0.065	80.17	0.232	0.539
P_3 x P_5	1861.14	5.17	1.921**	-0.077	80.00	-0.142	0.907
P_4 x P_5	1841.12	3.00	0.471**	-0.070	77.33	-2.068*	5.332**
Checks							
Nath Samrat 1133	1530.57	3.17	0.507*	0.018	91.83	2.296	2.341*
Surya	2032.55	4.17	1.341	1.107**	79.33	1.878	0.080
Mean	1482.23	3.798	1.000		80.12	1.000	
SE (±)	219.936	0.373	0.355		1.009	1.430	

Expression of unusual characters in ear shoot and tassel of maize

--Singh, NK; Devi, P; Mishra, P

Maize (*Zea mays* L.) is a monoecious species that produces only unisexual flowers in separate male and female inflorescences. It is one of the most important cereals, with the highest yield potential and diverse uses from staple food and feed to industrial products like starch and biofuels. It is strongly believed that maize is essential for global food security. Maize is largely grown under rainfed conditions where various abiotic and biotic stresses severely affect the genetic yield potential. A global climatic change is now considered to be underway and is expected to result in a long-term trend towards changes in environmental conditions. Congenial environmental seasons support optimal development, however, unfavouable environments influence the genetic architecture of the plant and reduce yield directly by affecting plant growth and development, and indirectly by modifying the normal plant phenotype. Unpredictability of weather conditions has occasionally resulted in many unusual expressions in plant characteristics in general, and ear and tassel characteristics in particular, in maize. Multiple ears on single nodes are one of the environmentally induced oddities widely reported in maize hybrids grown during 2006 in Iowa, Illinois, and Indiana. The expression of multiple ears in inbred lines, populations and experimental hybrids was also recorded in maize grown in the Tarai region of Uttarakhand, India during the monsoon season of 2007. The twin ear expression on