somes by searching for chromosomes with more than a single structural variation resulting from several breakages.

In this study, we used the variety Black Mexican Sweet Corn, homomorphic for a large terminal knob on the short arm, as the high-loss stock with B chromosomes to induce breakages in chromosome 9. This stock was used to pollinate a tester stock, homomorphic for a small terminal knob on chromosome 9 and homozygous for all four mutant genes, *yg2, c1, wx and bz1*. *yg2* produces yellow-green seedlings and plants. For statistical analysis, the variance of the recombination frequency between the loci was estimated by the inverse of the Fisher information index, and the confidence intervals were established by approximation with the normal distribution with 95% probability, and by the bootstrap technique with 5,000 simulations (Liu, 1998).

Plant number S-284-7 was a yellow-green exception with a deficient chromosome 9, which, when cytologically analyzed, had the following constitution for the chromosomic pair 9: one chromosome with a small terminal knob on the short arm, and the other knobless. Since the high-loss stock was homozygous for the large knob, the knobless chromosome represented, in fact, a very small deficiency. This plant also presented no pollen abortion. The ratio of *Wx* : *wx* kernels, corresponding respectively to the deficient and the normal chromosomes, in both male (232 *Wx* : 242 *wx*) and female (211 *Wx* : 213 *wx*) test crosses, was essentially 1:1. Genetic tests showed that although the deficiency was very small, *C1*, located at the 5th or 6th chromomere, carried out by the knobbed chromosome 9 of the high loss stock, was changed (mutated) to *c*, inactivated (paracentric inversion with one breakpoint inside the *C* or transposon insertion) or removed (internal deficiency), resulting in the unexpected expression of the recessive phenotype. In a test for the presence of a deficient chromosome 9, half of the resulting seedlings were white or green-white and the other half yellow-green or green-yellow-green striped. This demonstrates that the knobless chromosome 9 in plant S-284-7 is deficient for a very small terminal segment including the *Wd*. It also lacks *C1*, although it is present in the homozygous condition in chromosome 9 of the high-loss stock. Genetic evidence showed that *Bz1*, located at five map units to the right of *C1*, had not been deleted, but this was not a critical test for the internal deficiency hypothesis. Crossing over was therefore studied in the *Bz1*-*Wx* region, which is proximal to *C1*. The *Yg2*-deficient chromosome 9 (Df9) carried the dominant *Bz1* and *Wx* while the normal chromosome 9 (N9) contained the recessive alleles. A plant of Df9 *Bz Wx* / N9 *bz1 wx* constitution, pollinated by a *bz1 wx* tester homozygous for *C1*, supplied the results presented in Table 1. The variance of the recombination frequency (r) between *Bz1* and *Wx* and the confidence intervals (CI) are as follows: r estimation = 0.1698; CI (normal approximation) = 0.1445 - 0.1950; CI (bootstrap) = 0.1214 - 0.1922. The 16.98% of recombination found in the Df9/N9, originating from plant S-284-7, did not differ significantly from the control value of 18.9% observed in a related stock presenting *C1*. A somewhat better test for the hypothesis of internal deletion or paracentric inversion with one breakpoint inside *C1* was done by determining the percentage of recombination between *Wx* and the extremity of 9S, which is genetically marked because it is deficient for *Yg2*. The kernels from this cross were classified for *Wx* (Df9) and *wx* (N9) endosperm, and the resulting seedlings sorted as white or green-white striped (Df9) versus yellow-green or green-

Table 1. Percentage[1] of crossing over between *Bz* and *Wx* in plants heterozygous for Df S-284-7 (Df9 *Bz1 Wx*/N9 *bz1 wx*).

Phenotype	Number of kernels
Bz1 Wx	360
Bz1 wx	60
bz1 Wx	84
bz1 wx	344
Total	848
Crossing over (%)	16.98
Control	18.90

[1]Data from four years

Table 2. Percentage of recombination between *Wx* and the terminal deficiency in plants heterozygous for the S-284-7 deletion (Df9 *Wx wd*/N9 *wx yg2*).

Plant number	*Wx-wd*	*wx-wd*	*Wx-yg2*	*wx-yg2*	Total	Recombination (%)
S-1012-3	61	14	22	77	174	20.7
S-1012-4	37	5	7	65	114	10.5
S-1012-5	47	7	9	55	118	13.6
S-1012-8	65	17	15	65	162	19.7
Total	210	43	53	262	568	16.9
Control (S-996-7)	70	37	32	79	218	31.5

Wx-wd: white and green-white striped; *wx-wd:* white and green-white striped; *Wx-yg2:* yellow-green and green-yellow striped; *wx-yg2:* yellow-green and green-yellow striped

yellow-green striped (N9) phenotypes (Table 2). In family S-1012, the average frequency of recombination between *Wx* and the *Yg2* deficiency was 16.9% (Table 2). This value is approximately the same as the 16.98% recombination found between *Wx* and *Bz1* in comparable heterozygotes, indicating that no crossing over took place distally to *Bz1* in the deficient chromosome 9 of plant S-284-7. In plant S-996-7, heterozygous for a deficient chromosome 9, there was 31.5% recombination between *Wx* and the breakpoint in the deficient chromosome. The statistical analysis showed the following results: r estimation = 0.1690; CI (normal approximation) = 0.1381 - 0.1998; CI (bootstrap) = 0.1250 - 0.1866. Because the estimation for the control (0.315) is outside the estimation confidence intervals, one can conclude that there is a difference between the recombination percentage values of the analyzed material and the value of the control, with 5% probability. Since examination at pachynema of S-284-7 heterozygotes revealed no extensive deficiency, the low recombination value of 16.9% cannot be ascribed to a terminal deficiency in 9S, including the *C1* locus.

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Drought tolerant mutant induced by gamma-ray and sodium azide from maize calli

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In this study, our objectives were to develop drought-tolerant mutants and identify an optimum combination of γ ray with NaN_3 to treat embryonic calli derived from immature embryos in maize using the methods of Fu et al. (J. Sichuan Agric. Univ. 18:97-99, 2000; J. Northwest Sci-Tech Univ. Agric. For. (Nat. Sci. Ed.) 31:81-84, 2003). At present, information about workable dosages of gamma-rays and concentrations of NaN_3 to treat plant calli for mutation is limited. In rice, calli differentiation was enhanced with gamma (γ) ray at less than 30 Gy, but inhibited at higher than 40 Gy (Wang et al., Acta Agric. Nucl. Sin. 7:20-28, 1993). However, 1 kR, equivalent to 8.7 Gy of γ ray, was a suitable dosage to treat calli in wheat (Gao et al., Acta Agron. Sin. 20:18-25, 1994). For rice seed, the suitable dosage of γ ray and concentration of NaN_3 could be as high as 200 Gy and 2 mmol/L, respectively (Wang et

al., 1993). In this study, a combination with 20 Gy of γ ray and 1 mmol/L of NaN_3 was identified as suitable for mutation induction from maize calli (Table 1). The γ ray dosage in the present study was much higher than 0.1 kR or 0.87 Gy, the dosage used to mutate wheat anthers by Zheng et al. (Acta Bot. Sin. 35:121-128, 1993). Also, the NaCl concentration for mutant screening used in this study was twice as high as that used to screen mutated wheat anthers by Zheng et al. (1993). Haploid pollen in anthers is more responsive to γ ray treatment and NaCl screening than diploid embryonic calli.

Table 1. Survival percentage of maize calli treated with different doses of gamma-ray and NaN_3 on high osmotic medium.

NaN_3 concentration mmol/L	gamma-ray dosage (Gy)				
	0	10	20	30	40
0	0.33 (±0.04)	0.38 (±0.11)	1.04 (±0.06)	0.00 (±0.00)	0.00 (±0.00)
0.5	0.00 (±0.00)	0.24 (±0.05)	0.78 (±0.08)	0.00 (±0.00)	0.00 (±0.00)
1.0	0.00 (±0.00)	0.07 (±0.02)	1.43 (±0.07)	0.23 (±0.01)	-
1.5	0.12 (0.01)	0.34 (±0.13)	0.32 (±0.15)	0.06 (±0.03)	-
2.0	0.00 (±0.00)	0.00 (±0.00)	-	-	-

Maize embryonic calli derived from immature embryos of inbred line 18-599 were treated with gamma (γ) ray and sodium azide (NaN_3), and selected on high osmotic medium containing 1.0% NaCl for drought tolerance. Once plants were regenerated from the selected calli, they were evaluated for drought tolerance in artificial and natural conditions with parental 18-599 and known drought-tolerant line 81565 as checks. Anthesis-silking interval and grain yield were investigated as an estimate of drought tolerance.

Mutagenesis. Immature embryos of 1.5-2.5 mm in length were sampled from inbred line 18-599 13 days after pollination and cultured in the improved N6 medium for callus production. Line 18-599 is not only used as parent in many commercial hybrids, but is also suitable for tissue culture. Embryonic calli (type II) identified by the standard described by Armstrong and Green (Planta 164:207-214, 1985) were subcultured in the dark at 27 C for multiplication. The calli were irradiated with ^{60}Co γ ray at 10, 20, 30 and 40 Gy, respectively when they became stable after three subcultures, three weeks each. One week after stabilizing the cultures, they were treated with NaN_3 (pH3) at 0.5, 1.0, 1.5 and 2.0 mmol/L for 4 h. Relative growth rate used to determine the influence of mutation treatments with γ ray and NaN_3 on callus growth was recorded as [(callus weight after stabilizing cultures - callus weight before mutation treatments) / callus weight before mutation treatments] after stabilizing cultures for two weeks.

Mutant selection. The treated calli were then transferred to high osmotic medium containing 1.0% NaCl and cultured at 27 C twice, three weeks each. After stabilizing the high osmotic cultures for two weeks, these calli were transferred to differentiation medium for regeneration. The regenerated plantlets (M_0) grew in pots for hardening in the greenhouse for 2-3 weeks, and then in the field for selfing to produce M_2 seed. Drought stress was not applied to M_1 generation in the field because the plants were fragile, and the objectives in this generation were to produce enough seed for selection of target mutants and to observe morphological variation from the mutation treatments.

During the screening culture, most of the calli treated with gamma-ray and NaN_3 became brown and died on the high osmotic medium of 1% NaCl. After differentiation, regeneration and transplanting, 20 and 2 fertile plants were obtained from dosage combinations of 20 Gy of γ ray with 1 mmol/L of NaN_3 and 20 Gy of γ ray with 0.5 mmol/L of NaN_3, respectively. A few plantlets regenerated from several other dosage combinations, but failed to survive afterwards due to either poor growth or female sterility of the resulting plants.

The drought tolerance of mutated line 18-599M expressed from M_2 to M_4, as well as hybrids derived from the line, was significantly higher than parental 18-599 (Table 2) in various evaluations conducted in both artificial conditions and naturally water-stressed environments of four provinces (Sichuan, Ningxia, Guangxi and Hainan). Genetic polymorphism between 18-599M and 18-599 was found for 7 of 700 pairs of SSR primers listed at MaizeGDB. (Fig. 1). Among these polymorphic locations, the sequence amplified by SSR primer pair *phi080* located in chromosome bin 8.08 was identified as the 5'-end of glutathione S-tramsferase gene (*GST-1*) (Ulmasov et al., Plant Mol. Biol. 26:1055-1064, 1994). Glutathione S-transferase is one of the key enzymes involved in resistance to oxidation. The relationship of this enzyme with resistance to oxidative, osmotic, heavy metal stress and ultraviolet damage has been demonstrated (McGonigle et al., Plant Physiol. 124:1105-1120, 2000; Ulmasov et al., Plant Physiol. 108, 919-927, 1995). The expression of the glutathione S-transferase gene is up-regulated under drought stress in maize and transgenic *Arabidopsis* (Zheng et al., Plant Mol. Biol. 55:807-823, 2004), and its expression in wheat was up-regulated under polyethylene glycol simulated osmotic stress (Gallé et al., Acta Biol. Szeged. 49:95-96, 2005). More studies are needed for a molecular explanation of drought tolerance associated with genetic mutation in plants.

Nature of the mutations. Based on the sequence of the *GST-1* gene (GenBank accession number: M16900; Shah et al., Plant Mol. Biol. 6:203-211, 1986), we designed a pair of specific primers to amplify exon 1 of this gene: CACCCGATGCAACTTGCGTAGA[1] /GTTCCTACGCTTAGCCCAGAT. Three insertions, three dele-

Table 2. Comparison of anthesis-silking interval and grain yield between hybrids derived from mutant 18-599M and parental 18-599 (CK) under drought environments in three provinces (Sichuan, Ningxia and Hainan).

	Anthesis-silking interval (d)			Grain yield (kg/ha)		
	478	48-2	R15	478	48-2	R15
18-599M	2.63	3.12	2.46	4860*	6940*	6400
18-599 (CK)	2.52	3.56	2.32	4560	6760	6560

* Significantly (P<0.05) higher than its comparative commercial hybrid.

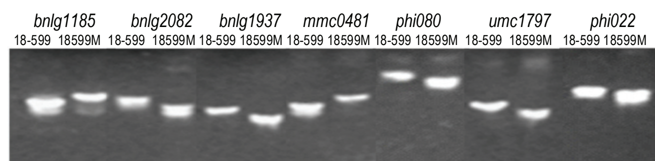

Figure 1. SSR polymorphism between mutated line 18-599M and parental 18-599. Genetic polymorphism amplified by 7 of 700 pairs of SSR primers between mutated line 18-599M and parental 18-599 showed that the genetic differences occurred in different locations of the genome from the mutagen treatments. For each pair of the primers, 18-599M is located in the left lane and 18-599 in the right lane.

tions and seventeen base substitutions were found in exon 1 of the glutathione S-transferase gene by sequence alignment. Base substitutions from A to T at 158 bp (A to T) and (C to G) at 162 bp changed the encoded amino acids from methionine to leucine and from serine to tryptophan, respectively (Fig. 2). The insertion of $(AGAGG)_5$ between the 86 and the 87 bp of 18-599 (Fig. 2) was probably responsible for the larger amplified product in 18-599M (Fig. 1).

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18-599M  CNNATNNNNC ACCCGATGCA ACTTGCGTAG AGAGTTGGGC GCAGAGAATC CCCAAG-
CAAA CAAACAGGGT AGAGGGAGAG GAGAGGAGAG GAGAGGAGAG  100
18-599   CNNATNNNNC ACCCGATGCA ACTTGCGTAG AGAGTTGGGC GCAGAGAATC CCCAGG-
CAAA CAAACAGGGT AGAGGGAGAG GAGAGG                  86

18-599M  GAGAGGAGAG GTTGGGTCTG GGACACCATG GCTCCGATGA AGCTGTACGG
GGCGGTGATG TCGTGGAACG TGACGAGGTG CGCAACGGCG CTGGAGGAGG  200
18-599              TTGGGTCTG GGACACCATG GCTCCGATGA AGCTGTACGG
GGCGGTGTTG TGGTGGAACG TGACGAGGTG CGCAACGGCG CTGGAGGAGG  175

18-599M  CTGGCTCCGA CTACGAGATC GTGCCCATCA ACTTCGCCAC CGCCGAGCAC AA-
GAGCCCCG AGCACCTCGT CCGCAACGTA CCGTACCTTC CCGATCCTCC  300
18-599   CTGGCTCCGA CTACGAGATC GTGCCCATCA ACTTCGCCAC CGCCGAGCAC AA-
GAGCCCCG AGCACCTCGT CCGCAACGTA CCGTACCTTC CCGATCCTCC  275

18-599M  CTCTCCCTCT CCCGTTGTTG TTGTTGTTGT TTGTTTGTTC TTCCCGGTAA TTGAAAT-
GCA GCGTCCGTTC CGTTCGCGCG AAGGGGTGGC GTGGCGCTGC  400
18-599   CTCTC      CCGTTGTTG TTGTTGTTGT TTGTTTGTTC TTCCCGGTTA TTGAAAT-
GCA GCGTCCGTTC CGTTCGCGCG ATGGGG     GTGGCGCTGC  365

18-599M  AGTCGGCCTA TCGTCGACGG CCGATCTGAC TCCCCTCTAG CGCG******
******ATGG GTTTTGTTTC ACGGCAAACT GGGGG*TTTC GGATTTAAGG  487
18-599   AGTCGGCCTA TCGTCGACGG CCGATCTGAC TCCCCTCTAG CGCTTAGCCA
CCCGTTAAGG GTTTTGTTTC ACGGCGAACT GGGGGGTTTC GGATGTAAGG  465

18-599M  CTGCGGTTTC GCGGAGGGAA TCCAAAATTG ACAAATAAGG GAATCTGTTC
CATACTGTAA ATGGTAAAAT TAGTTACAAG ATTATAAGAA ATGGACTAA*  586
18-599   CTGGGGTTTC GCGGAGGGAA TCCAAAACTG ACAAATAAGG GAATCTGTTC
CATACTGTAA ATGGTAAAAT TAATTACAAG ATTATAAGAA ACGGAATCGT  565

18-599M  *******CCGTAGGTTGTTTCAGATCTGGGCTAAGCGTAGGAAC
623
18-599   GGAATTTCTGTAGGTTGTTTCAGATCTGGGCTAAGCGTAGGAAC
609
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Figure 2. Sequence alignment of exon 1 of *GST-1* gene between 18-599M and 18-599. Three insertions, three deletions and seventeen base substitutions in exon 1 of glutathione S-transferase gene in 18-599M compared to parent allele, 18-599. The black rectangle shows the start codon of the coding sequence.

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[1]The first primer is the same as for *phi080* SSR (MNL Editor note).