Alleles of *pink scutellum1* with no visible kernel phenotype

--Stinard, PS; Jackson, JD

The Maize Genetics Stock Center has been maintaining two independently isolated seedling mutants (*peach-albino-mutable*-87-2209-30* and *peach-albino*-N1983B*) with a unique peach-tinged albino phenotype. Because of their similar phenotype, tests of allelism were performed and these two mutants were found to be allelic. Rescued seedlings from viviparous alleles of *pink scutellum1* are described as being white with a pink flush. *ps1* mutant alleles are blocked in the production of carotenoids and the pink color is due to the accumulation of lycopene. Many *ps1* mutant alleles are also viviparous due to ABA deficiency. Several dormant alleles of *ps1* have been described which produce pink seedlings with varying degrees of greening (Faludi-Daniel et al., Acta Agron. Hung. 16:1-6, 1967; Bai et al., Genetics 175:981-992, 2007). However, these dormant *ps1* alleles produce kernels with visibly altered endosperm carotenoids that are pinkish in color, also due to the accumulation of lycopene. The *peach albino* mutants do not have visibly altered endosperm carotenoids and mutant kernels are indistinguishable from nonmutant kernels in a *Y1* background. Nevertheless, due to the similar mutant seedling phenotype, allelism tests were performed between the *peach albino* mutants and a viviparous *ps1* allele (*ps1-8205*). From the allelism test crosses, ears were obtained that segregated for pink endosperm kernels with dormant embryos. Seedlings grown from pink kernels had the seedling phenotype of their respective *peach albino* parent (Figure 1). It is interesting to note that although the double heterozygote *peach albino*/*ps1* kernels retained the embryo dormancy of the *peach albino* parent, they retained the endosperm carotenoid expression of the *ps1* parent. We conclude that the *peach albino* mutants are dormant alleles of *ps1* that have a unique nonmutant endosperm phenotype.

Figure 1. Seedlings grown from allelism test cross ears of *peach-albino*-N1983B* (middle row) and *peach-albino-mutable*-87-2209-30* (right) with *ps1-8205*, pink kernels planted. Note the sectors of greening on the plants on the right. They are not revertant sectors but rather represent an allele-specific epigenetic phenomenon. On the left are albino *lemon white1* seedlings for purpose of comparison.

Two point linkage data for *Og1* and *oy1* on chromosome 10

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Classic maize genetic linkage maps (e.g., Mutants of Maize, Neuffer et al., Cold Spring Harbor Laboratory, New York, 1997) show a separation of 4 centiMorgans between the *og1* and *oy1* loci on chromosome 10. These data appear to be based on indirect mapping with respect to other markers; no direct mapping between these loci has been reported in the literature. Since dominant *Og1* mutant alleles condition green and yellow/yellow-green striping and there exist dominant mutant alleles at the *oy1* locus that condition yellow-green plants, the possibility exists that *og1* and *oy1* may represent the same locus. We conducted direct mapping experiments between a dominant *Og1* mutant and a recessive *oy1* mutant to try to get at this question.

Homozygous *Og1 Oy1* (*Old gold1* single mutant) plants were crossed to homozygous *og1 oy1* (single mutant *oil yellow1*) plants and the resulting double heterozygotes were backcrossed by a homozygous *og1 oy1* tester. Kernels from testcross ears were planted and the resulting plants scored for *Og1* and *oy1*. 206 green seedling/Old gold striped parental type plants (*Og1 Oy1*) and 186 oil yellow seedling/green parental type plants (*oy1 og1*) were observed. No double mutant *Og1 oy1* plants were observed. Five potential double nonmutant green seedling/green plants were self-pollinated and evaluated one additional generation to confirm genotypes; all five turned out to be single mutant parental class *Og1 Oy1* plants. Thus no crossovers were obtained from a total of 397 plants scored, indicating a separation of less than 0.25 +/- 0.25 centiMorgans. These are not enough data to draw a definitive conclusion, but these two loci are certainly tightly linked if not identical.

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Genetic evidence of an unexpected kind of chromosome 9 aberration induced by the B chromosome in maize

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The maize B chromosome has been associated with induction of late knob replication in A chromosomes resulting in bridge-breakage, loss of acentric fragments and production of terminal-deficient chromosomes. B chromosomes have no effect upon the viability of the organism, inasmuch as they are not essential for growth and development. The B chromosome has been so far best studied in maize, and causes several interesting genetic effects. One of these effects, discovered by Rhoades et al. (1967), is the so-called “high-loss phenomenon”, in which B chromosomes interact with the knobs of A-complement chromosomes, inducing breakage. Since different kinds of chromosome aberrations, besides the single terminal deficiency, have been genetically described and were not included in the Rhoades and Dempsey hypothesis, they demand more investigation aimed at a better understanding of the whole effect of the B chromosome in inducing breakage in chromosomes of the A-complement. With this purpose in mind, this study was conducted in order to genetically analyze an aberrant plant with deficiency induced by B chromo-

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-yellow 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.

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

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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