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Screening for genetic control of intragenic recombination by monosomic analysis

I recently determined that intergenic recombination in the sh-wx region of chromosome 9 is consistently, significantly lower in monosomic 4 plants (11.2% + 0.8%) and monosomic 8 plants (17.1% ± 0.9%) than in diploid control plants (23.0% ± 0.8%) (Weber, submitted for publication). It appears to be unchanged in monosomic 7 plants (21.8% ± 1.8%), thus, monosomy per se does not alter intergenic recombination. Since consistent, significant alterations in intergenic recombination were found to occur in specific monosomic types, I have initiated a study to determine if intragenic recombination can also be altered by monosomy of specific maize chromosomes. Initial results of this study are presented in this paper.

To study intragenic recombination, one must analyze large populations because the recombinational frequencies are very low. These large populations can be readily analyzed using the elegant system developed by Nelson (1959, Science 130:794). This system uses the waxy (wx) locus on chromosome 9 of maize. Since the phenotype of a pollen grain is determined by its genotype and because massive numbers of pollen grains are available from a single plant, the populations necessary for such a study can be efficiently analyzed using relatively simple procedures.

The r-X1 deficiency in maize induces chromosomal nondisjunction during the megagametophyte divisions after meiosis, producing large numbers of monosomes, trisomes, double monosomes, double trisomes, and even triple monosomes (Weber, 1973, Theor. Appl. Genet. 43:167). R/r-X1 plants (from Satyanarayana) were backcrossed as female parents by plants homozygous for the wx-90 allele (from O. Nelson) of the wx locus for six backcrosses to establish an inbred line carrying both the r-X1 deficiency and the wx-90 allele.

These r-X1; wx-90 plants were crossed as females by a second inbred, Mangelsdorf's multiple chromosome tester (from Satyanarayana), which bears a recessive gene on each chromosome (bm2; lg; a; su; pr; y; gl; j; wx; g). As the r-X1; wx-90 line carries a dominant gene corresponding to each recessive marker in Mangelsdorf's tester except for wx, the appearance of a recessive phenotype in the F_1 indicates the loss of a chromosome carrying the corresponding allele from the maternal parent. Such a plant would be monosomic for that chromosome. For a more detailed description of the production of monosomics with the r-X1 deficiency, see Weber, 1973, Theor. Appl. Genet. 43:167.

The wx allele in Mangelsdorf's tester is presumably the standard recessive allele of the wx locus designated wx-C, for Cornell (Nelson, 1968, Genetics 60: 507), therefore this allele will be designated wx-(C). All F_1 progeny from the above cross (r-x1; wx-90×Mangelsdorf's tester) are very highly isogenic except for aneuploidy because they are the result of a cross between two inbred lines. All F_1 progeny also carry the wx-90 allele on one chromosome 9 and wx-(C) on its homolog.

F_1 progeny from the above cross were planted in the field and specific monosomic types were selected. Plants monosomic for chromosomes 2, 7 and 10, as well as diploid plants, have been recovered and analyzed to date. Tassel samples from monosomic and diploid plants were fixed in 70% ethanol. Twenty-four anthers were placed into a Virtis microhomogenizer stainless steel cup, cut apart with a scissors, and homogenized for 1 min in 0.5 ml of I_2-KI solution as described by Nelson (1968). The homogenate was then strained through two layers of cheesecloth onto the surface of an 80 x 100 mm slide. A 45 x 50 mm coverglass was placed on the preparation.

The population of normal pollen grains on each slide was estimated by counting twenty 1 mm^2 areas on each slide and multiplying this value by a constant. Only plump pollen filled grains were counted. Each slide was then scanned, and each Wx pollen grain was counted. The Wx frequencies in the two inbred lines were 1.8×10^{-5} for wx-(C) and 0.8×10^{-5} for wx-90. These values are in the range of those reported by Nelson (1968). The mean of these values was subtracted from the frequency of Wx pollen grains from monosomic and diploid plants to compensate for back mutation and suppressor mutation. The corrected values are presented in Table 1.

Table 1. Corrected frequencies of Wx pollen from diploid and monosomic plants.

Plant type	Estimated No. of gametes	Wx X 10^{-5}	$\bar{X} \pm SE$
Diploid (3 plants)	115,540	66	
	93,260	89	
	172,800	84	79.7 ± 6.99
Monosomic 7 (4 plants)	72,375	86	
	84,380	66	
	120,710	51	
	83,250	64	66.8 ± 8.31
Monosomic 2	73,130	34	
Monosomic 10	124,760	88	

The corrected frequency of Wx pollen in diploid control plants is similar to the value (102 X 10^{-5}) reported by Nelson (1962, Genetics 47:737) for recombination between wx-C and wx-90; therefore, the wx-(C) allele in the Mangelsdorf's tester stock and wx-C are a similar distance from wx-90. It is likely that wx-C amd wx-(C) are the same allele.

Estimates of corrected Wx frequencies within the diploid and monosomic 7 classes were found to be homogeneous by means of a χ^2 test for homogeneity of binomial proportions, and a pooled frequency was calculated for each class. Neither the frequency in the monosomic 10 plant nor the pooled frequency in the monosomic 7 plants was significantly different from the pooled frequency in diploids. Thus, monosomy per se does not alter intragenic recombination between these two alleles.

However, the corrected Wx frequency from the monosomic 2 plant was highly significantly different from that of the diploids ($p<.0001$). More data are obviously needed, but this indicates that intragenic recombination in this monosomic 2 plant was sharply reduced from that which occurred in the controls or the other monosomic types analyzed. It will be extremely interesting to determine if a comparable reduction will be found in intergenic recombination in monosomic 2 plants, or an increase in trisomic 2 plants.

We would like to express our appreciation to Funk's Seeds International for generously providing summer nursery space in Shirley, Illinois, and winter nursery space in Molokai, Hawaii, in which material utilized in these studies was grown.

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