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GENETIC ANALYSIS OF THE R-MARBLED ALLELE IN MAIZE (*ZEA MAYS* L.)

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ABSTRACT

The assumption that the pattern allele R-marbled of maize represents a compound form with more than one component was verified by recombinational analysis, using a recessive g r-g tester stock. R-mb gave rise to self-coloured exceptions in testcrosses. Progeny testing of these kernels indicated that R-mb has two components — a self-colour component is responsible for anthocyanin pigmentation, while a controlling element, designated as Mb, inhibits the Sc action during the clonal development of the aleurone. Through the recovery of g-Sc cases from the test crosses, it was ascertained that recombination is possible between Sc and the flanking golden plant colour marker g, with the loss of the controlling element.

Key words: R-marbled, compound form, recombination, maize.

The pattern alleles at the R locus in maize (R-marbled, R-stippled and R-Navajo) produce unique patterns of anthocyanin pigmentation in the aleurone layer of endosperm. R-marbled (R-mb) confers large patches of irregularly shaped, intensely coloured sectors on a colourless background. Unlike R-stippled (R-st) and R-Navajo (R-nj), which have received considerable attention from maize workers, only limited literature is available on R-mb. Earlier reports indicated that recovery of reverse mutations of marbled to self-coloured phenotype was more frequent than that of self-coloured from stippled [1, 2]. This observation led to the assumption that R-mb represents a compound form with more than one component. The present investigation aims to elucidate the constituent components responsible for producing the marbled pattern through recombinational analysis.

MATERIALS AND METHODS

The R-mb and g r-g genetic stocks used in the present study were originally obtained from the University of Wisconsin and University of Missouri, U.S.A., respectively. These genes were eventually transferred to a well-adapted background stock, Stock 2 developed from the breeding inbred, CM 105. The genotypic constitution of Stock 2 was pl, A1, A2, C1,

C2, r-g, Bz1 and Bz2; it did not carry any transposable element system like Activator (Ac), Dotted (Dt) or Suppressor-mutator (Spm).

The R-mb seedlings are normally green but they occasionally form small amounts of anthocyanin. The homozygous recessive g r-g tester stock, carrying the genetic marker g at 14 map position proximal to the R locus, is characterized by the absence of colouration in any seed or plant part. The proximal marker g conditions golden plant colour of plants at the flowering stage and golden silks. The stock also carried an unlinked white endosperm (y) marker on chromosome 6 to aid in the exclusion of kernels resulting from cross-contamination by foreign pollen. Lack of a suitable distal marker that can give consistent results precluded the possibility of a three-point testcross. The scope of this study is therefore limited to ascertaining whether recombination is possible between Sc and g, with corresponding loss of the proposed controlling element at the R locus.

The G R-mb homozygous lines were crossed to the homozygous recessive g r-g stock. The testcross (G R-mb/g r-g x g r-g) progeny were classified at the seed stage for aleurone colour and examined for non-parental kernels. The testcross ears yielded mostly marbled and colourless kernels and occasionally self-coloured (Sc) exceptions. The presumed self-coloured (R-sc) variants were progeny tested to verify their origin and segregation behaviour. If the self-coloured kernels originated due to recombination between the constituent components of the R-mb gene, the progeny should segregate for self-coloured and colourless kernels only. On the contrary, coloured seeds in the testcross ears arising from pollen contamination would reveal on selfing, extraneous characters carried by the contaminant pollen and segregation for R-mb; thus, exceptional cases due to contamination can be excluded. Cobs that segregated only for self-coloured and colourless phenotypes were isolated as presumed intralocus recombinants. These kernels were grown and the plants so obtained were scored for the golden plant colour constitution. Plants homozygous for g marker were pale yellow with golden silks. If reversion from R-mb to R-sc was accompanied by crossing over in the proximal region, the variant plants should exhibit the golden phenotype. True variants should also have green anthers. Chi-square test was applied to find out if the observed segregation for coloured : colourless fits the expected ratio of 3 : 1.

RESULTS AND DISCUSSION

Screening of 10,799 kernels from 56 testcross ears yielded 35 nonparental, self-coloured kernels that might be recombinants. Of these, selfed cobs could be obtained from only 28 plants. Three of these twenty eight self-coloured exceptions produced marbled kernels as well in the selfed cobs; mottled kernels were also noticed which indicated that the endosperm genotype of these kernels is R-mb R-mb R, arising out of contamination in the F_1 generation by R-carrying pollen. In the remaining twenty five crosses, selfed ears

segregated for self-coloured and colourless kernels only. The colourless kernels showed only white endosperm (y) (Table 1). A record was also kept for the plant colour : normal (+)

Table 1. Kernel phenotypes obtained after selfing of the plants derived from self-coloured (Sc) variant seeds recovered from G R-mb/g r-g x g r-g testcrosses

Plant number	Self-coloured	Colourless	Marbled	Total kernels	Plant colour	χ^2 (3:1)[a]
1	103	21	—	124	+	4.30*
2	155	26	83	264	+	—
3	137	47	—	184	+	0.03
4	74	23	—	97	+	0.09
5	224	81	—	305	g	0.67
6	314	93	—	407	+	1.00
7	48	17	—	65	+	0.05
8	33	18	—	51	+	2.88
9	94	32	—	126	+	0.01
10	152	48	—	200	+	0.11
11	279	99	—	378	+	0.29
12	23	21	—	44	+	0.98
13	153	55	—	208	+	0.23
14	205	68	—	273	g	0.00
15	227	80	—	307	+	0.18
16	88	52	—	140	+	11.01*
17	221	61	—	282	+	1.71
18	252	—	118	370	+	—
19	130	—	83	213	+	—
20	290	93	—	383	+	0.11
21	77	23	—	100	g	0.21
22	102	35	—	137	+	0.01
23	89	34	—	123	g	0.46
24	78	19	—	97	+	1.52
25	82	32	—	114	+	0.57
26	115	42	—	157	+	0.26
27	278	85	—	363	+	0.49
28	232	67	—	299	+	1.07

Plant colour: + wild type green, g—golden.

[a]Tested against the expected 3:1 ratio for coloured:colourless.

*Significant at P = 0.05.

or golden (g). Four out of the 25 cases (plant Nos. 5, 14, 21, 23) had golden plant colour and green anthers. The segregation of coloured:colourless in these four cobs also fitted the expected ratio of 3:1. These g-Sc cases therefore appear to be the products of the cross-over events at R-mb with recombination between flanking marker (g) and the self-colour component (Sc). This represents only one strand of the crossover products that could be phenotypically detected. The other strand was not identifiable and was included in the colourless parental class.

The results suggest that the two components, Sc and Mb are physically separable through recombination. The expectation of recombination event between g and Sc (R) is 14%. Hence, out of 25 self-coloured exceptions, the expected frequency of golden plants is 25 x 0.14 = 3.5, which is very close to the observed number (four plants). Therefore, the four g-Sc cases recovered are most likely from recombination between g and Sc. It is also plausible that the controlling element (Mb) is proximal to Sc.

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