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Genetic structure of the *R-Navajo* allele in maize, *Zea mays* L.

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Summary. Mutational and recombinational analyses carried out with the *R-nj* allele in maize to elucidate the genetic mechanism involved in unique pattern formation and origin of occasional self-coloured kernels in this stock revealed that *R-nj* represents a complex with two closely linked discrete components. The self-colour (*Sc*) component is responsible for anthocyanin production and the navajo (*Nj*) component regulates the time of onset and termination of pigment synthesis restricting the pigmentation to the crown region of the kernel. The probable gene order in the *R* region of the *R-nj : Illinois* isolate is: *G-Sc-Nj-K*.

Key words: Genetic structure – *R-nj* Allele – Mutation – Recombination

Introduction

Some of the members of the multiple allelic *R* series in maize produce in combination with other complementary genes unique patterns of anthocyanin distribution on the aleurone layer and offer excellent material for studying differential gene action. *R-Navajo* (*R-nj*) produces a solid anthocyanin pigmented patch in the crown region of the kernel and also at the embryo tissues, silks and anthers. It was observed that the *R-nj* allele infrequently mutates to a fully self-coloured form (*R-sc*) suggesting that *R-nj* is a compound allele having at least two members (Kumar and Sarkar 1978, 1986).

The present investigation was directed at attempting to identify and isolate the constituent genetic elements at this locus responsible for anthocyanin production in one part of the aleurone and its simultaneous repression in another part of the tissue, and to elaborate the topographical arrangement of the elements in *R-nj* through mutational and recombinational analyses.

Materials and methods

Genetic stocks used were: *R-nj : Illinois* (with linked markers: Golden (*g*), about 14 map units proximal to *R*; and *K*, a large heterochromatic knob, 35 map units distal to *R* that normally shows approximately 1–2% recombination with *R*), *m R-nj* (= *R-njMp*, mutable *R-nj*), *r-g* (absolutely no anthocyanin pigmentation in aleurone and any plant tissue), in a uniform genetic background stock 2. Unlinked factor used to detect pollen contaminants was (*y*) conditioning white endosperm.

Mutational analysis

The *m R-nj* stock was selfed, crossed to the *r-g* tester and the F_1's were backcrossed to the *r-g* tester. The frequencies of *R-nj* and self-coloured mutants that arise by germinal transposition of *Mp* away from the *R* locus, were estimated on selfed *m R-nj*, F_1's and testcross ears. The self-coloured kernels were progeny tested.

Recombinational analysis

The *G R-nj K* line was crossed to the *g r-g k* tester line and the F_1's were backcrossed to the tester. The backcross progenies (*G R-nj K/g r-g k* × *g r-g k*) were classified for aleurone colour and examined for nonparental kernels. Chi-square values for 75: 25 testcross ratio of *R-nj* vs colourless were calculated.

Genetic analysis of the self-coloured exceptions arising in the testcross ears

The self-coloured exceptions were grown. The ears on these plants were self-pollinated to discriminate between intra-allelic recombinants and contaminations. If the self-coloured seeds originated from recombination at the *R-nj* allele, the progeny ears should show segregation for self-coloured and

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colourless kernels only. On the other hand, coloured kernels in the testcross arising from pollen contamination may produce, on selfing, foreign characters and segregation for *R-nj* from these exceptional cases would indicate contamination origin. All the ears that segregated self-coloured and colourless phenotype, were isolated as presumed intra-allele recombinants. The flanking marker constitution of the self-coloured plants were established by scoring in the field for the normal or golden plant colour. Scoring for *K* on the selfed ears was done by counting the coloured and colourless kernel populations and by observing the normal or distorted ratio.

Table 1. Results from progeny testing of self-coloured kernels obtained from the testcross (*m R-nj/r-g*×*r-g*)

Ear no.	Self-colour	*R-nj*	Spotting on crown	Total coloured	Colour-less	Total
1	186	–	–	186	71	257
2	170	–	–	170	66	236
3	197	–	–	197	68	265
4	183	–	–	183	64	247
5	191	–	–	191	66	257
6	219	–	–	219	180	399
7	361	10	25	396	–	396

Results

Mutational analysis

The primary observations supporting the compoundness of *R-nj* gene would be regular occurrence of stepwise events inferring that two or more units are mutating independently. It was thought that the *m R-nj* allele would be useful in such studies as it was observed to throw occasional self-coloured (*Sc*) mutants (Kumar and Sarkar 1978, 1983). The *m R-nj* allele is made up of the *R-nj* gene and a modulator, *Mp,* integrated at or near this gene. Transposition of *Mp* from this site in the aleurone tissue leads to the formation of coloured patches or spots. Release of *R-nj* gene from *Mp* in the germ cells would result in the manifestation of stable *R-nj* phenotype (Greenblatt and Brink 1962). Infrequent occurrence of stable *Sc* kernels may be interpreted as due to removal of a *R-nj* component, *Nj,* which controls restriction of anthocyanin to the crown region only, during *Mp* transposition. This proposition was verified by studying the progeny from the *m R-nj* stock.

Selfed *m R-nj* kernels on 63 ears yielded 11 self-coloured and 158 *R-nj* kernels out of a total of 6,840. The frequencies for self-coloured and *R-nj* work out to be 1.61×10^{-3} and 23.10×10^{-3}, respectively. The 11 self-coloured mutants were grown and progeny testing could be done in 10 plants only. The ten self-coloured mutants gave 3:1 ratio for self-coloured vs spotted crown phenotype. Few kernels with stable *Navajo* phenotype were also obtained. The self-coloured kernels presumably arose from *m R-nj* either by inactivation or transposition of *Nj* along with *Mp.*

In the ears from F_1 between *m R-nj* and *r-g* tester self-coloured kernels were not obtained. Out of 556 kernels in the limited crosses made, 23 were *R-nj* and none showed self-coloured phenotype.

The scoring of testcross (*m R-nj/r-g*×*r-g*) kernels on 33 ears yielded seven self-coloured and 93 *R-nj* kernels out of a total of 4,246. Coloured vs colourless segregated into 1:1 ratio. The frequencies for self-colour and stable *R-nj* work out to be 1.65×10^{-3} and 21.90×10^{-3}, respectively. Out of seven self-coloured mutants, six segregated self-coloured and colourless phenotypes whereas one segregated self-coloured, *R-nj* and spotted crown phenotypes (Table 1). It is difficult to explain the seventh case as it is not segregating for colourless kernels which are expected in one-fourth of the seeds. One possibility, that there was out-contamination with *R* pollen at the testcross stage, fails to account for the paucity of *R-nj* and *m R-nj* phenotypes which together should have added upto about 100 kernels in this selfed ear. One individual (sixth case) on selfing gave a 9:7 ratio for coloured: colourless suggesting its contamination origin. Other five individuals showed a 3:1 segregation for self-coloured and colourless kernels. All of them presumably arose from the *R-nj* gene either by inactivation or transposition of *Nj* component or crossing over to isolate *Sc* component of *R-nj.*

Recombinational analysis

Occasional appearance of self-coloured kernels in the *R-nj* stock led to the assumption that *R-nj* may represent a compound form with more than one component and that mutation of *R-nj* to *R-sc* may be due to loss of one component through mutation or recombination. This assumption was sought to be confirmed through recombination studies using *g* and *K* flanking markers. The preferential segregation frequency for *K 10* is known to be 0.70 (Rhoades 1942) and that of *R* in *K 10* carrying stock is 0.75 (Sarkar 1972). The chi-square values for the majority of the individual ear segregations were not significant for the expected testcross segregation ratio of 75 coloured:25 colourless confirming the preferential segregation frequency of 0.75 for the *R* locus in the *K* carrying stock. A total of 51,006 kernels from 346 testcross ears (*G R-nj K/g r-g k* ×*g r-g k*) were screened and 61 self-coloured kernels were isolated. These may represent or include the suspected recombinant cases. Attempts were made to determine the mode of origin of these 61 exceptional cases.

Table 2. Progeny test of suspected intra-allele recombinants obtained from *G R-nj K/g r-g k* × *g r-g k* testcross

Ear no.	Self-colour	*R-nj*	Colour	Total	χ^2-value[a]
1	68	28	–	96	0.89
2	102	33	–	135	0.03
3	4	130	–	134	–
4	346	103	–	449	1.02
5	290	93	–	383	0.11
6	306	99	–	405	0.07
7	182	67	–	249	0.23
8	56	33	–	89	6.93**
9	222	121	–	343	19.32**
10	77	37	–	114	3.38
11	24	15	6	45	–
12	137	94	–	231	30.34**
13	130	83	–	213	22.16**
14	46	24	–	70	3.22
15	26	34	–	60	32.08**
16	252	118	–	370	9.37**
17	–	102	9	111	16.89**
18	–	80	20	100	1.33
19	–	126	25	151	5.74*
20	–	252	43	295	17.09**
21	–	261	38	299	24.09**
22	–	77	22	99	0.41
23	–	126	25	151	5.74*
24	–	56	11	67	2.63

[a] Tested against a 3:1 ratio for the coloured vs colourless
*, ** Significant at 5% and 1% level, respectively

Table 3. Progeny test of suspected intra-allele recombinants obtained from *G R-nj K/g r-g k* × *g r-g k* testcross

Ear no.	Self-colour	*R-nj*	Colour-less	Total	χ^2-value[a]	Genotype in respect of *y* marker
1	74	–	23	97	0.09	Yy
2	29	–	12	41	0.39	Yy
3	47	–	15	62	0.02	Yy
4	310	–	104	414	0.00	Yy
5	105	–	36	141	0.02	Yy
6	295	–	116	411	2.28	Yy
7	314	–	115	429	0.75	Yy
8	315	–	95	410	0.73	Yy
9	276	–	95	371	0.07	YY
10	93	–	31	124	0.00	Yy
11	280	–	100	380	0.35	Yy
12	300	–	90	390	0.77	yy
13	289	–	104	393	0.45	yy
14	227	–	77	304	0.02	yy
15	244	–	62	306	3.66	yy
16	204	–	58	262	1.14	yy
17	82	–	32	114	0.57	yy
18	155	–	52	207	0.00	yy
19	154	–	51	205	0.00	yy
20	23	–	11	34	0.98	yy
21	97	–	47	144	4.48*	yy
22	104	–	22	126	3.82*	yy

[a] Tested against a 3:1 ratio for the coloured vs colourless
* Significant at 5% level

Table 4. Outside marker constitution of presumed intra-allele recombinants

Sl. no.	Normal or golden	Silk	Anther	Glume
1	+	–	–	–
2	+	+	+	–
3	+	–	–	–
4	+	–	–	–
5	+	–	–	–
6	+	+	–	+
7	+	–	+	+
8	+	–	+	+
9	+	–	+	+
10	+	–	+	+
11	+	+	+	+

+: Normally green or anthocyanin-pigmented; –: golden or non-pigmented

Genetic analysis of the self-coloured exceptions arising in the testcross ears

Sixty one self-coloured kernels obtained from the testcross *G R-nj K/g r-g k* × *g r-g k* were grown in the field. Out of these selfed ears were obtained from only 46 plants. The results of the progeny test are presented in Table 2 and 3. Of the 46 self-coloured exceptions tested, 24 yielded *R-nj* kernels on selfing (Table 2). Out of these 24, 16 cases segregated for self-coloured and *R-nj* phenotypes. These may be discarded as obvious out-contaminations of the F_1 plants by *R* carrying pollen. Eight ears showed segregation for *R-nj* and colourless phenotypes. These may also be accounted for as arising from mistake in classification as the phenotypic expression of these *R-nj* kernels showed colouration of the entire endosperm surface during classification. Twenty two plants yielded ears segregating for self-coloured and colourless kernels (Table 3). The contamination marker, *y* (white endosperm), was included in both the *R-nj* and the *r-g* tester stock. When these ears were examined, *Y-y* segregation was noticed in 11 cobs indicating that these too arose from out-contamination. The remaining 11 cases were homozygous *y y* suggesting that these might be genuine cases arising from recombination event at the *R-nj* gene. These 11 cases (Sl. No. 12–22, Table 3) showed a 3:1 segregation for coloured and colourless kernels indicating absence of heterochromatic knob *K* from the vicinity of the *R* locus. All the 11 plants were non-golden (green) and *k*.

Detailed records for anthocyanin colour in anther, glume and silk were also kept for all the exceptional cases (Table 4). Out of the 11 plants four showed absence of anthocyanin in anther, glume and silk, If we assume that anther, glume and silk colour are conditioned by independent genes (Sarkar et al. 1975), at least four of the 11 cases seem to be the products of

crossover events at the *R-nj* gene with recombination of the flanking markers.

Discussion

The cells of the entire aleurone layer of *R-nj* kernels are genetically alike but the pigment production is restricted only to the crown of the kernel. The assumption that *R-nj* represents a compound form consisting of at least two components – one the self-colour, *Sc*, responsible for pigmentation of the whole kernel, and the second, an inhibitor designated *Navajo* (*Nj*), responsible for the restriction of aleurone colour to the crown portion of the kernel to produce the pattern was verified through mutation studies using mutable *R-nj* gene. Genuine self-coloured mutants were obtained from selfed *m R-nj*^{v} and testcrossed (*m R-nj*/*r-g*×*r-g*) progenies. These mutants presumably arose from *R-nj* gene either by inactivation or transposition of *Nj* component or crossing over to isolate *Sc* component of *R-nj*.

The compound structure of *R-nj* was confirmed through recombination studies using flanking markers. Eleven genuine self-coloured recombinants were obtained. For their origin out-contamination could be ruled out. Almost all of them segregated coloured and colourless kernels in 3 : 1 ratio. The flanking marker constitution of these self-coloured recombinants was *G* and *k* suggesting that all were crossover between *G* and *K*. This indicates that the *Sc* component of *R-nj* is proximal and *Nj* component is distal. Their order with reference to *G* and *K* is *G Sc Nj K*. The recombination event may be diagrammatically represented as follows:

$$\begin{array}{cccc} & \overbrace{}^{R\text{-}nj} & & \\ G & Sc & Nj & K \\ \hline g & r & & k \end{array} \qquad \begin{array}{ccc} G & & k \\ \hline g & r\ Nj & K \end{array}$$

(1) (2)

Of the two crossover products expected, only strand (1) is recovered in the testcross as it produces phenotypically indentifiable recombinant (the self-colour) in the progeny; the reciprocal event, strand (2) isolating *Nj* is not identifiable and is included in the colourless parental seed class.

Four of these 11 cases showed absence of anthocyanin in anther, glume and silk. This suggests that the four cases are the products of crossover events at the *R-nj* gene with recombination of the flanking markers.

Some speculations may be made as to the nature of the proposed *Nj* component responsible for restriction of aleurone colour to certain areas of the kernels. Available information indicates that it is an inhibitor gene similar to the *C-I*. It regulates the timing of *R* gene action in the endosperm so that anthocyanin production is restricted to the crown region only. Normally anthocyanin pigments first appear at the crown region and gradually spread towards the base of the kernel. Late initiation of pigment appearance in *R-nj* kernels (Styles et al. 1973) can not fully account for the vast range of variability in *R-nj* expression (Kumar and Sarkar 1986). By assuming a restriction of anthocyanin production to a short period of time most of these variations can, however, be explained. Hence, the regulatory role of the *Nj* component may be explained by assuming that *Nj* allows *Sc* gene to produce anthocyanin for a brief period after which *Nj* inactivates the function of *Sc*. The *Sc* gene of *R-nj* may not be identical with *S* of *R-r* as in the *R-nj*/*R-r* heterozygote, self-colour kernels result. The difference may be due to the presence of a receptor for *Nj* at the *Sc* gene which conditions *Sc* to respond to the singals from *Nj*.

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