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CONTROLLING ELEMENTS INDUCING MUTATIONAL CHANGES AT THE *PAL* LOCUS IN *ANTIRRHINUM MAJUS*

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Abstract

In *Antirrhinum majus* the *pal-rec-low-o* is a repressed state of the *pallida-recurrens* allele. In heterozygous condition, especially with *pal-tub pal-tub* tester, its mutation frequency increases from few tiny spots to very clear sectors of mutability on a recessive colourless background. This particular evocation of the *pal-rec-low-o* allele is considered to be dependent on the presence of a regulatory element *Pr*. In the absence of such a regulatory element, the *pal-rec-low-o* exhibits stable expression owing to the effect of a repressor Rp residing at or near the locus. However, *pal-tub* regulatory element could be inactive, when inactive, can be made transactive by introducing a fresh regulator which may segregate at meiosis.

Introduction

The *pal-rec-low-o,* an unusual allele of the *pallida* Series, exhibits stable expression in homozygous condition, except for rare late mutant spots. But when crossed with the recessive tester strain *pal-tub pal-tub* (also stably colourless), the mutation frequency increases giving very clear sectors of mutability on a recessive colourless back ground. This particular evocation of *pal-rec-low-o* instability with *pal-tub pal-tub* tester was considered to be related to paramutation of *R* in maize (Brink, 1973).

The present study of the *pal-rec-low-o* showed that the change in *pal-rec-low-o* gene activity is dependent on the presence of an independently located *Pr* element, contributed by the *pal-tub* tester strain. In the absence of such a regulatory element, the *pal-rec-low-o* exhibits stable expression when homozygous owing to the effect of a 'repressor' element residing at or near the locus (Aslam, 1987). However, in several cases it was found that the *pal-tub* coming through a phase of heterozygosity has changed. Evidence has been presented to show that when *pal-tub* regulatory element is inactive, it can be made transactive by introducing a fresh regulator into the genome which can segregate at meiosis. An appropriate association of an active *Pr* regulatory element with the repressor element of the 'original' low line, can result in variegation presumably through excision of the repressor element.

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Materials and Methods

The main experimental material, *pal-rec-low-o* was established at Leeds from *Antirrhinum majus* seeds imported from India. Plants derived from the original material showed varying degrees of variegation. But two plants turned out to be exceptionally low in mutability showing one or two tiny spots on corolla lobes in each plant. Seed obtained from those two exceptionaly low in mutability plants provided a homozygous stock for *pal-rec-low-o* (original) and has been maintained at Leeds in its original homozygous condition by selfing. Another genetic strain, *pal-tub pal-tub* tester used in the present study was originally obtained from the John Innes Institute several years ago and has been maintained at Leeds. The Antirrhinum stock from both the sources, which exhibit different phenotypes corresponding to respective genotypes are given in Table 1.

Quantitative estimates of floral instability were obtained by scoring individual flowers against a standard scale. The scale consisted of 0-8 classes. On this scale, class 0 represented flowers with no mutant spots, and fully coloured phenotypes fell in the highest class; the intervening classes represented the intermediate grades of instability. Scoring was however, checked regularly by complete spot counts of representative classes with the aid of a microscope.

Table 1. Phenotypic description of some of the genetic lines used in the study.*

Genotype	Flower Phenotype
pal-tub pal-tub (tester)	Acyanic except for pigmented ring around the base of corolla tube.
pal-rec-low-o pal-rec-low-o (original)	Almost acyanic except for occasional magenta spots on corolla lobes, maintained by selfing.
pal-rec-low-nc pal-rec-low-nc (near colourless)	Uniformly low line with occasional magenta spots, derived from the 'original' through crosses.
pal-rec-low-o pal-tub	Variegated corolla with a solid magenta ring at the base of the flower.
pal-rec-low-nc pal-tub	Variegated corolla with a magenta ring at the base of the flower tubes.
pal-rec-low-act pal-rec-low-act	An established uniformly high mutable line.

*None of the lines carry *St*, a known modifier (Harrison & Fincham, 1968)

Results

The hybrids (*pal-rec-low-o pal-tub*) with very low mutability, when backcrossed to plants homozygous for *pal-rec-low-o*, only produce non activated *pal-rec-low pal-tub* plants. This indicated that even the tester line is not homogeneous and probably there is something in the original *pal-rec-low-o* which suppresses the instability of *pal-rec* gene (Aslam, 1987).

To substantiate this hypothesis, two extremely low heterozygous individuals (45-453-10 and 45-453-6) of the type mentioned above were selfed to obtain *pal-tub* segregants. Homozygous *pal-rec-low-o* (original) were used to see whether these segregated *pal-tub pal-tub* plants could, infact activate *pal-rec-low-o* gene. No symptoms of mutability were found except for a few late mutant spots, characteristics of *pal-rec-low-o* (original). This is in contrast to the control crosses in which *pal-tub pal-tub* tester were used, where several plants became activated (Table 2). This difference in *pal-rec-low-o* gene activation between *pal-tub pal-tub* tester and segregants could be explained on the basis of the following possibilities: (a) the segregated *pal-tub pal-tub* plants might have

Table 2. Heterozygous plants of *Antirrhinum majus* (*pal-rec-low pal-tub*) with low scores were selfed and the *pal-tub pal-tub* segregants were crossed with the homozygous *pal-rec-low-o* (original).

Cross No.	Cross pal-tub pal-tub x pal-rec-low-o pal-rec-low-o (segregants)			Plants scored	Means ± S.D.
1	45-625-8	x	45-591-1	14	0.35 ± 0.15
2	45-625-12	x	45-589-6	21	0.25 ± 0.13
3	45-625-6	x	45-589-11	21	0.25 ± 0.18
4	45-627-1	x	45-589-6	21	0.20 ± 0.10
5	45-625-6	x	45-589-6	24	0.25 ± 0.18
6	45-625-6	x	45-591-1	20	0.35 ± 0.15
7	45-625-12	x	45-589-6	24	0.45 ± 0.18
8	45-627-1	x	45-589-11	24	0.30 ± 0.12
9	45-627-1	x	45-591-1	24	0.25 ± 0.10
			Total	193	0.29 ± 0.00 mean
#10	45-591-1	x	45-584-20	20	3.10 ± 0.33
#11	45-589-15	x	45-584-3	20	3.00 ± 0.35
			Total	40	3.05 ± 0.00 mean

#Control crosses (*pal-rec-low-o pal-rec-low-o* x *pal-tub pal-tub* tester).
Statistical analysis: $t = 46.27$; $df = 9$; ($P < 0.001$)

inherited a factor, *Rp* which sustains *pal-rec-low-o* in a repressed condition; (b) it is possible that *pal-tub pal-tub* tester plants themselves contain a regulatory factor which specifically activates the repressed *pal-rec-low-o;* on that basis *pal-tub pal-tub* segregants which failed to activate would be lacking in that element. This element would be referred to as *Pr* ; (c) also, the segregants were heterozygous for *Pr* and *Rp* elements since they produce two types of plants.

The above ideas are further supported from the *pal-tub pal-tub* plants derived from an heterozygous individual, 45-453-16 which was characterized by mutability class 2.0 shifting. Only two categories of *pal-tub pal-tub* segregants were tested when it was selfed. One category of segregants such as 45-627-4 produced about 50% of reasonably high mutables (class 3.00 ± 0.60) when crossed to homozygous *pal-rec-low-o* while the rest of them were found to be colourless. Closer examination of the latter revealed a few late mutant spots (Table 3). The second category of *pal-tub pal-tub* segregants such as 45-627-1 extracted from the same mutable individual produced plants of only "near colourless" phenotypes (Table 2).

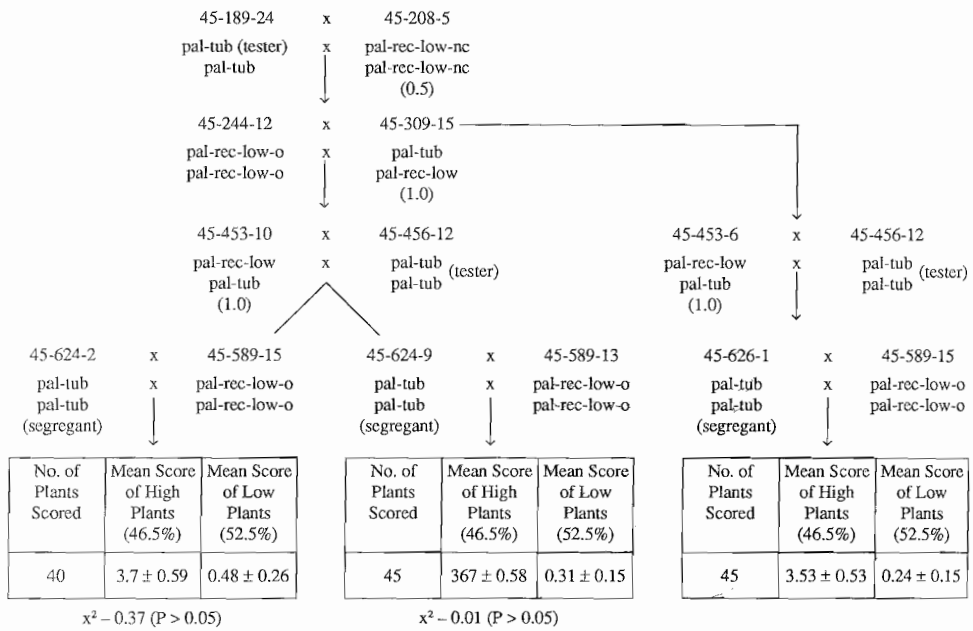
From these findings, it appears that the first category of *pal-tub pal-tub* segregants was carrying two independent factors, *Pr* and *Rp*, responsible for *pal-rec-low-o* gene activation and repression respectively: hence, when segregated at meiosis they produced two types of progeny. But the second category of segregants was either homozygous for the 'repressor' (*Rp*) factor or lacked both a repressor and an active *Pr* regulatory element, and hence were inert.

Table 3. Effect of *pal-tub pal tub* segregants of the *Antirrhinum majus* obtained following a cross between pal-rec-low pal-tub (class 0.5) and pal-tub pal-tub (tester), on homozygous *pal-rec-low-o*.

Cross No.	*pal-tub pal-tub*	Cross x	*pal-rec-low-o pal-rec-low-o*	F1 Score of high plants ± S.D. #	F1 Score of low plants ± S.D. #
1	45-624-2	x	45-589-15	3.94 ± 0.84 (9)	0.48 ± 0.26 (7)
2	45-624-9	x	45-589-13	3.84 ± 0.82 (13)	0.31 ± 0.15 (8)
3	45-227-4	x	45-591-1	3.00 ± 0.60 (5)	0.48 ± 0.29 (6)
4	45-626-1	x	45-589-15	3.78 ± 0.75 (14)	0.20 ± 0.12 (7)
5	45-624-9	x	45-589-15	3.50 ± 0.35 (9)	0.20 ± 0.10 (15)
6	45-626-1	x	45-589-13	3.28 ± 0.32 (14)	0.24 ± 0.10 (10)
			Mean	3.55 ± 0.36 (64)	0.31 ± 0.13 (53)

Statistical analysis: X^2 (for high and low mutables (1:1) = 1.03 (P> 0.05)

number shown in brackets indicates the number of plants scored.



No. of Plants Scored	Mean Score of High Plants (46.5%)	Mean Score of Low Plants (52.5%)
40	3.7 ± 0.59	0.48 ± 0.26

$x^2 - 0.37$ ($P > 0.05$)

No. of Plants Scored	Mean Score of High Plants (46.5%)	Mean Score of Low Plants (52.5%)
45	367 ± 0.58	0.31 ± 0.15

$x^2 - 0.01$ ($P > 0.05$)

No. of Plants Scored	Mean Score of High Plants (46.5%)	Mean Score of Low Plants (52.5%)
45	3.53 ± 0.53	0.24 ± 0.15

Fig. 1. Derivation of *Antirrhinum majus pal-tub pal-tub* plants which segregated out following a cross of an extremely low heterozygous mutable 45-430-10 with *pal-tub pal-tub* tester; and their crosses with homozygous *pal-rec-low-o* to show that an initially inactive *pal-tub* made trans-active by introducing a fresh *pal-tub*, is now segregating.

Figure 1 supports the hypothesis that non *pal* factors are involved. Assuming that the heterozygous low mutable (45-453-10) used carries an *Rp* (repressor) then, when such a mutable was crossed with *pal-tub pal-tub* tester, some segregants would obtain *Rp* from the low mutable and an active *Pr* regulatory element from *pal-tub* tester stock, to produce bifactorial segregants. Such segregants produce two types of progeny when crossed to homozygous *pal-rec-low-o*, indicating independent segregation of two at meiosis. Alternatively, if it is assumed that the particular low mutable (45-453-10) lacked an active *Pr* regulatory element in its genome, with the introduction of a fresh one through *pal-tub-pal-tub* tester it was made trans-active, segregating out at meiosis and thus producing high and extremely low individuals.

Interestingly enough, *pal-tub pal-tub* plants extracted from very highly activated *pal-rec-low-act* lines showed a higher activation of the *pal-rec-low-o* gene than that produced by *pal-tub pal-tub* tester; when crossing *pal-tub pal-tub* segregants, the low "original" line produced progeny with a 4.35 ± 0.45 score; *pal-tub pal-tub* tester produced only class 2.70 ± 0.35. The difference of almost two classes is the highly significant (Table 4).

Table 4: Effect of *pal-tub pal-tub*, extracted from *pal-rec-low-act pal-tub* mutables on *pal-rec-low-o pal-rec-low-o* (original).

Cross No.	Cross (*pal-tub pal-tub* x pal-rec-low-o pal-rec-low-o)			Plants scored	Means ± S.D.
1	45-594-4	x	45-589-15	21	5.00 ± 0.30
2	45-594-8	x	45-589-13	21	4.85 ± 0.25
3	45-594-4	x	45-589-15	25	4.20 ± 0.35
4	45-594-6	x	45-589-15	25	4.16 ± 0.40
5	45-594-8	x	45-589-15	25	4.25 ± 0.40
6	45-594-9	x	45-589-15	25	4.60 ± 0.30
7	45-594-13	x	45-590-13	25	3.55 ± 0.40
8	45-594-13	x	45-589-15	25	4.15 ± 0.35
			Total	192	4.35 ± 0.45
9	45-591-1	x	45-584-20[a]	20	3.00 ± 0.35
10	45-589-1	x	45-584- 3[a]	20	3.00 ± 0.35
11	45-589-6	x	45-584-20[a]	20	2.40 ± 0.40
12	45-589-11	x	45-584-20[a]	20	2.40 ± 0.45
			Total	80	2.70 ± 0.35

a: Control crosses (*pal-rec-low-o pal-rec-low-o* x *pal-tub pal-tub* tester)
Statistical analysis: $t = 6.26$; $df = 10$; ($P < 0.01$).

Discussion

The *pal-rec-low-o* is a special repressed state of the *pal-rec* gene. This particular repressed state showed various grades of mutability when made heterozygous with *pal-tub* tester strain. Within F_1 individuals reversion towards the original state was also found (Sastry, 1976), and this phenomenon was related to paramutation of *R* in maize, a system originally described by Brink (1973).

In present investigation, a search for genetic basis resulted in finding two categories of elements, the regulator *Pr*, contributed by the stably recessive *pal-tub* and a repressor *Rp*, by the 'low original'. It has already been demonstrated elsewhere (Aslam, 1987) that the *pal-rec-low-o* gene function is determined by the activity of the *Rp* element which is resident to the 'original' line. On making plants of 'near colourless' phenotype heterozygous with *pal-tub* tester, the factor originally responsible for repression presumably moves away (unstable event) from the locus of the gene and can be segregated with *pal-tub*. An appropriate association of such a stabilizing factor with another factor of destabilising nature may result in plants with sectorial appearance.

The *pal-rec-nc pal-tub* hybrids with very low scores were crossed back to *pal-rec-low-o*; the resulting heterozygotes only produced plants with low phenotype, indicating loss of *pal-tub* capacity to induce a mutational change in activity of *pal-rec-low-o* gene. In the light of these observations, the activity of a *pal-tub* in association with *pal-rec-low-o* can be criticised. Initiated with *pal-rec-low pal-tub* plants with very low mutability scores, no evocation is either due to (a) loss of sensitivity of *pal-rec-low* to undergo changes, or (b) *pal-tub* losing its 'ability' to induce the change. Crossing with fresh *pal-tub pal-tub* tester showed that *pal-rec-low* still can undergo the change. The *pal-tub pal-tub*, on the other hand has in many cases failed to induce the change in homozygous *pal-rec-low-o* plants.

On the basis of such a specific genetic control of mutability, observations on *A. majus* can be campared with maize systems. For instance, McClintock (1956) found that in the absence of *Ac* (a regulatory element), no changes affecting gene action occurred and stability of gene expression was exhibited as long as *Ac* was not present. In many other cases described by McClintock (1962) and Peterson (1970) a mutable locus originaly closely associated with the controlling element loses its capacity to mutate autonomously; the locus itself becomes stable, showing either no mutability or very low levels of gene expression. This may still, however, mutate in response to the presence of a controlling element elsewhere in the genome. From this McClintock (1965) concluded that the initially autonomously mutable locus has become dependent for its mutability on an external regulator element only because of the transposition of the regulator to elsewhere in the genome, leaving the receptor alone at the mutable locus.

In *A. majus* the *pal-tub* regulatory functions in some cases could be of heterozygous nature (active and inactive-inducing effect) corresponding to cyclic changes in the activity of the element (Aslam & Sastry, 1979). Such an inefficiency of the *pal-tub* regulatory element, *Pr*, was observed in several cases (Fig. 1). However, the activity of an initially inactive *Pr* regulatory element can temporarily be restored if an active regulator is introduced which may segregatge at meiosis. These observations reflect some similariaties with bacterial systems of gene control, with special reference to *Insertion Sequences* (*IS* elements), when *IS 2* reduces operon expression on integration in a particular orientation (active phase), and when it is in opposite orientation (inactive phase), genes located down-stream are expressed constitutively (Nevers & Saedler, 1977). Where as *IS 1* behaves like *Rp* element and reduces operon expression when integrated in either orientation.

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