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Isolation of a new paramutagenic allele of the *sulfurea* locus in the tomato cultivar Moneymaker following in vitro culture

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Abstract. A new allele, SC148, of the *sulfurea* locus in *Lycopersicon esculentum* was detected in a line derived after repeated selfing of plants that had been regenerated from tissue culture. Like the original *sulf* mutant, SC148 displayed two mutant phenotypes: green-yellow speckled plants in which the *sulf*^{vag} allele is present and pure yellow plants homozygous for the *sulf*^{pura} allele. Although the mutant alleles are recessive to wild-type, an unpredictable number of variegated and pura plants appeared in F_1 progenies that had been derived from crosses between SC148 and wild-type tomato plants. The presence of the wild-type *sulf*$^+$ allele in these variegated heterozygotes was demonstrated using a cytological marker that is linked to *sulf*. It is concluded that the mutant *sulf* allele of SC148, imposes its variegated expression state on the wild-type *sulf*$^+$ allele present in *sulf*$^+$/*sulf*^{vag} heterozygotes. This behaviour, known as paramutation, has also been described for the original *sulf* allele. The SC148 allele, however, seems to induce changes at an earlier stage in development. The analogy of this paramutagenic system to dominant position effect variegation in *Drosophila* is discussed.

Key words: *Sulfurea* – Tomato – Paramutation – Dominant position effect variegation

Introduction

A puzzling genetic phenomenon, termed paramutation, involves the unusual interaction of two alleles when brought together in a heterozygote. In this interaction one allele, that is said to be paramutagenic, imposes its expression state on the other allele. This change is heritable because the altered expression state is maintained in the next generation. The resulting shift in phenotype, which often occurs from wild-type to mutant, is observed either directly in the heterozygote or in its offspring. Paramutation was first noticed for the rabbit ear rogue mutation of *Pisum sativum* in 1915 (Bateson and Pellow). Another example is the *sulfurea* mutant in tomato which was recovered after X-ray treatment of seeds of the variety Lukullus (Hagemann 1958, 1969). Two distinct mutant phenotypes, both recessive to wild-type, were documented: *sulf-variagata* recognizable by the appearance of yellow/green speckled leaves and *sulf-pura* with yellow/white cotelydons. The homozygous pura plants die in the seedling stage but can be rescued for genetic analysis by grafting the seedlings onto wild-type plants.

Both the *variegata* and *pura* allele were found to be paramutagenic as judged from the proportion of *sulf*$^+$/*sulf*^{vag} and *sulf*$^+$/*sulf*^{pura} heterozygotes developing variegated leaves and branches on the otherwise green plants. Upon selfing, an excess of variegated progeny was obtained which was proportional to the extent of variegation on the parent, indicating that the alteration of the wild-type allele was heritable. Hagemann (1969) compared the level of paramutagenicity of the *sulf*^{pura} and *sulf*^{vag} alleles in crosses with various tomato lines. The variable results indicated that two groups of alleles existed. Within the *sulf*^{pura} group, the *sulf*^{pura} alleles induced variegation in 0.5–100% of the F_1 (*sulf*$^+$/*sulf*^{pura}) plants, whereas in the *sulf*^{vag} group the maximum level was 12%. Remarkably, in hybrids with *L. hirsutum* and *L. pennellii* the level of paramutagenicity did not exceed 2% (Hagemann 1969).

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Other cases of paramutation, summarized by Brink (1973) and later by Hagemann and Berg (1977), involved loci in tomato (*C-6*), *Oenothera* (*cruciata*), *Celosia* (*ap*), *Malva* (*laciniata*), *Antirrhinum* (*deficiens* and *nivea*) and maize (*R* and *B*). Molecular investigations, although thusfar limited to only three cases of paramutation, suggest that the underlying mechanisms might be diverse (Krebbers et al. 1987; Dooner and Robbins 1991). In an experiment that evaluated the effectiveness of somaclonal variation (van der Bulk et al. 1990) a plant was recovered that carried variegated sectors. In the present study we show that this new mutant is an allele of the *sulfurea* locus of tomato and that, like the *sulfurea* allele isolated previously, this mutant allele is also paramutagenic.

Materials and methods

Plant material

The *sulfurea* mutant SC148 was recovered in an experiment designed to assess somaclonal variation in tomato plants that had been regenerated from leaf explants (van der Bulk et al. 1990). The selfed progenies (R_2 lines) of more than 900 regenerated plants of the tomato cultivar Moneymaker were screened for variant phenotypes. In a relatively large number of these R_2 lines a chlorotic semi-dwarf phenotype designated "type 57" was detected (van der Bulk et al. 1990). This "57" dwarf phenotype was transmitted to the progeny though no true breeding lines could be obtained. In an R_4 line derived from repeated selfing of the "57" dwarf type, 2 out of 21 plants (plant 900333 and 900334) developed variegated sectors on their leaves. The progeny of plant 900333 consisted of ten wild-type plants, while the progeny of plant 900334 segregated into 11 wild-type plants, two plants with variegated sectors and one uniformly variegated plant. This uniformly variegated R_5 plant, designated SC148, was further analysed. Variegated plants were not observed among the 23 R_2 and 19 R_3 plants derived from the initial R_2 line.

The original *sulfurea* mutant was recovered by Hagemann (1958) in the $X_2 (= M_2)$ generation following X-ray treatment of seed of the tomato variety Lukullus. To determine whether the SC148 allele is paramutagenic, one variegated R_6 plant (selected from the selfed progeny of SC148) was crossed to the cultivars Condine Red and Red Cherry, and to the genotypes GT (a tomato-mosaic-virus-resistant pure line, kindly provided by De-Ruiter seeds Bleiswijk, The Netherlands) and 2s-iso, a tomato line which carries an extra chromosome consisting of two heterochromatic short arms of chromosome 2, kindly provided by Dr. C. M. Rick (Ramanna et al. 1985). The phenotype of the seedlings were scored 3–4 weeks after sowing.

Cytological techniques

The length of the satellite on chromosome 2 was determined in meiotic pachytene cells using the cytological techniques described previously (Ramanna and Prakken 1967).

Results

Morphological variation among plants regenerated from cells or tissues is a common phenomenon and is known as somaclonal variation (Larkin and Snowcroft 1981). One such example is the newly isolated mutant SC148 (Fig. 1) whose green speckled phenotype is very

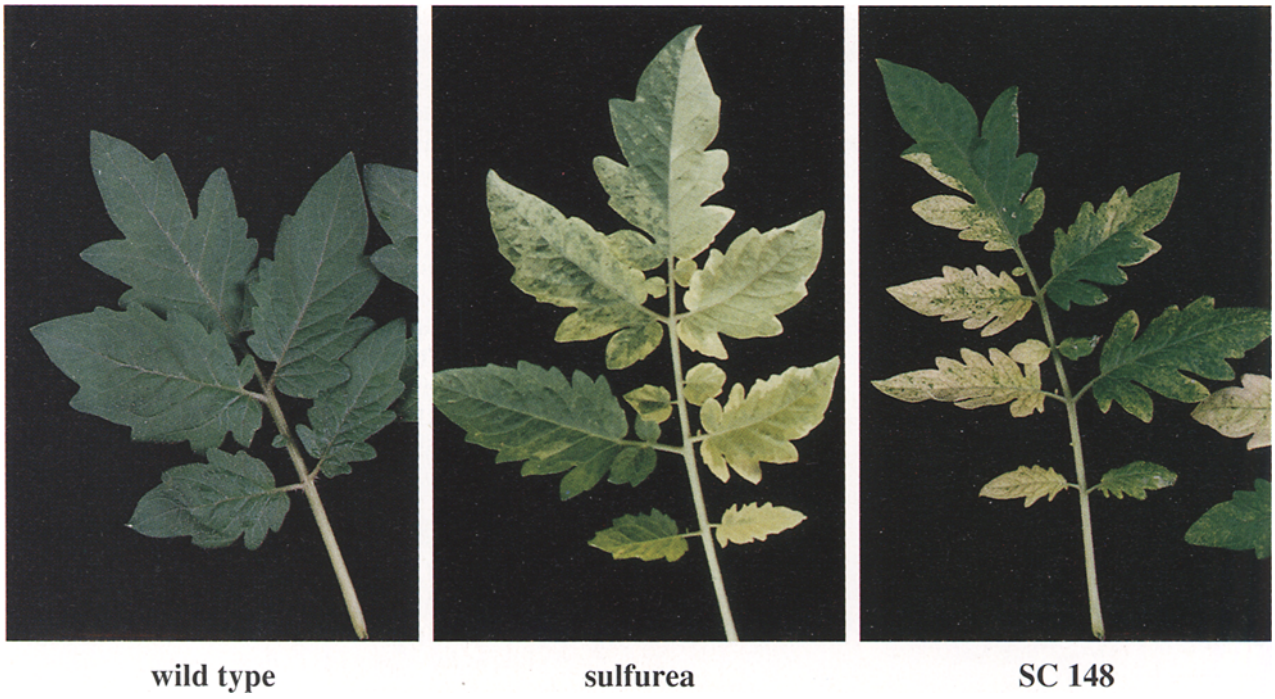

Fig. 1. Phenotype of the tomato mutants *sulfurea* and SC148

similar to the *sulfurea* mutant previously isolated by Hagemann (1958). Like the original *sulfurea* mutation, the selfed progeny of SC148 segregated in a non-Mendelian ratio into variegated and yellow seedlings (puras), with the latter dying at the seedling stage (Table 1). On testing the new mutant for genetic complementation with the original *sulf* locus, the F_1 showed a clear mutant phenotype, which, under the assumption that the mutation is recessive (see below), strongly suggests that the new mutant is an allele of the original *sulf* locus (Table 1).

As the mutant SC148 was identified among a population of 11 wild-type plants, the mutation is likely to be recessive to wild-type. This hypothesis was further tested by crossing the mutant to various wild-type tomato lines. The majority of the F_1 seedlings displayed a wild-type green phenotype, suggesting that the SC148 mutation was indeed recessive. However, as plant development proceeded, a variable number of green seedlings formed variegated sectors (Table 1). In addition, homozygous *pura* plants were found among the F_1 plants. The number of mutant offspring (variegated and pura) ranged from 25% for crosses with Red Cherry to nearly 50% for crosses with the 2s-Iso line. These observations indicate that, like the original *sulfurea* mutant of Hagemann, the SC148 allele is paramutagenic, changing the phenotype of the wild-type-locus into *variegata* or *pura* in somatic cells.

To confirm that the wild-type *sulf*$^+$ locus was indeed present in the variegated hybrids, the genotype of the variegated *sulf*$^+$/SC148 hybrids was determined using a cytological marker linked to the *sulf* locus. As the *sulf* locus is located on chromosome 2 (near or within the heterochromatic region bordering the centromere) and no crossing-over was observed between the satellite on the short arm of chromosome 2 and the centric heterochromatin, the *sulf* locus is thought to be absolutely linked to the satellite (Hagemann and Snoad 1971). The length of the satellite varies significantly among tomato genotypes (Ramanna and Prakken 1967; Hagemann and Snoad 1971). A rather short satellite is present in the SC148 mutant as in its parental genotype Moneymaker whereas the satellite of Condine Red is 5–6-fold longer (Fig. 2). Thus, the long satellite provides a useful diagnostic marker for the wild-type *sulf*$^+$ allele in crosses between SC148 and Condine Red. As shown in Fig. 2 the long satellite was present in SC148/Condine Red heterozygotes with a variegated phenotype, indicating that the wild-type *sulf*$^+$ allele was also present. From this result it is concluded that the wild-type *sulf*$^+$ allele indeed changed to variegata in SC148/Condine Red heterozygotes.

The results described sofar, indicated that the level of paramutagenicity, as determined by the percentage mutants present among the F_1 population, depended on the tomato line to which the SC148 was crossed. Hagemann (1969) attributed differences in paramutagenicity to the spontaneous appearance of variant alleles. The formation of new alleles is unlikely to explain the observed variation among F_1 populations because all crosses were made on the same SC148 plant. It is difficult to envisage that this plant passed on different alleles in every cross. Therefore, it was concluded that the variation is likely to reflect a genetic background effect on paramutation. In contrast, genetic background effects were not responsible for the variation in paramutagenic activity of the SC148 allele observed among plants from one particular F_1 population (some plants are green, other become variegated), because all F_1 plants should be of the same genotype. Apparently, the paramutagenic activity depends on other unknown factors.This was also inferred from the variable proportion of mutants detected in the F_2

Table 1. Segregations into green, variegated and pura plants in selfed, F_1, and F_2 progenies of SC148

Cross (female × male)	Phenotype of parents	Plant generation	Number of plants			% Mutant* (vag + pura)
			Green	Variegated (Vag)	Pura	
SC148 selfed	Variegated	I_1	–	71	13	100.0
SC148 × Sulfurea	Vag × vag	F_1	–	56	12	100.0
SC148 × Red Cherry	Vag × green	F_1	535	162	16	25.0[a]
SC148 × GT	Vag × green	F_1	123	52	11	33.9[b]
SC148 × Condine Red	Vag × green	F_1	545	266	56	37.1[b]
SC148 × 2s-Iso	Vag × green	F_1	583	542	26	49.3[c]
SC148 × Condine Red	Green	F_2	47	28	15	47.8[a]
SC148 × Condine Red	Green	F_2	18	72	3	80.6[b]
SC148 × Condine Red	Green	F_2	58	12	27	40.2[a]
SC148 × Condine Red	Green	F_2	73	21	0	22.3[c]

* Within F_1 and F_2 populations, a different letter (a, b or c) indicates a significant difference in the number of mutants when tested with a χ^2 test ($P < 0.05$)

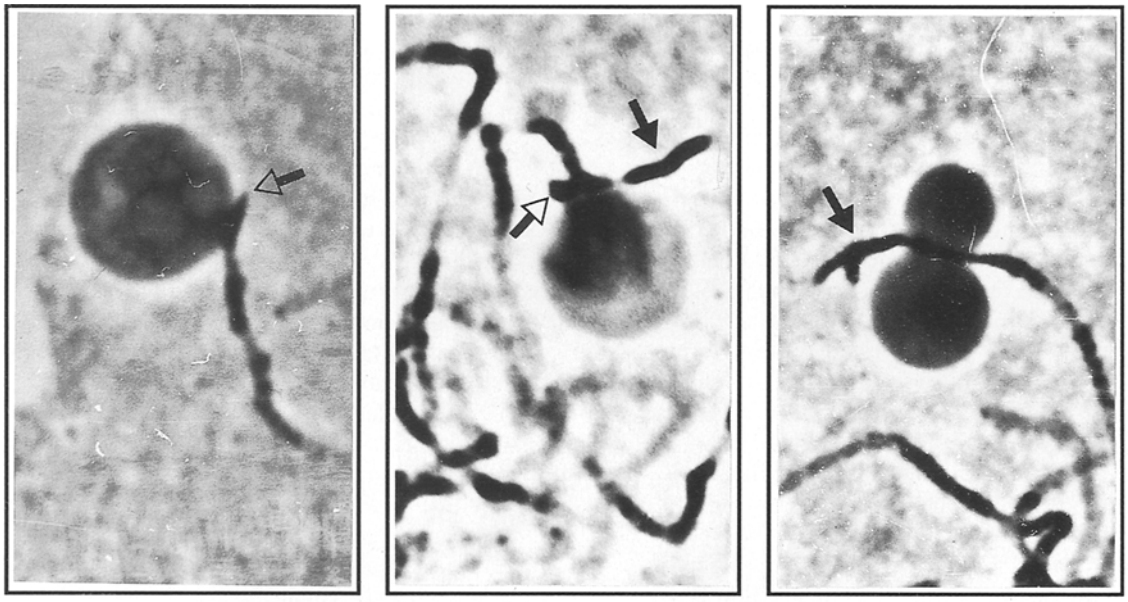

Fig. 2A–C. Light microscope images of the satellites of chromosome 2 in pachytene cells of Moneymaker (**A**), a Condine Red/SC148 heterozygote (**B**) and Condine Red (**C**). Note the short satellites in Moneymaker (*open arrowhead*), the long satellites in Condine Red (*bold arrowhead*), and the long and short satellite in the heterozygote

populations that had been derived from four SC148/Condine Red hybrids with a green phenotype (Table 1). These hybrids are thought to be genetically identical as the parental lines are homozygous. Yet, the proportion of mutants detected in their selfed progenies ranged from 22 to 81% (Table 1). In principle, a *sulf*$^{+}$/*sulf*^{vag} heterozygote produces upon selfing 25% variegated (*sulf*^{vag}/*sulf*^{vag}) plants and 50% *sulf*$^{+}$/*sulf*^{vag} heterozygotes that have the potential to become variegated through the action of the *sulf*^{vag} allele. Thus, the proportion of variegated offspring varies between 25 and 75% when the expression of the wild-type *sulf*$^{+}$ allele was altered in none or all heterozygotes, respectively. Among the four F_2 progenies tested, we found one example with approximately 25% mutants, indicating that the SC148 allele was not triggered to become paramutagenic. The other extreme progeny consisted of 81% mutant plants, a percentage that is not significantly different from 75%. This might also indicate that the original F_1 plant, although green, carried homozygous *sulf*^{vag}/*sulf*^{vag} sectors that apparently contributed to the offspring. The other two F_2 progenies consisted of an intermediate number of mutants, which was consistent with the expected number taking into account that in the F_1 population of this particular cross 37% of the plants became variegated (Table 1). Taken together, it is concluded that it is difficult to assign the level of paramutagenicity to a specific cause. Genetic as well as random factors could influence the level of paramutagenic activity of the SC148 allele. It also can not be excluded that new alleles differing in paramutagenic activity have arisen, as was suggested previously by Hagemann (1969).

Discussion

A new *sulfurea* mutant, SC148, was isolated following in-vitro regeneration of leaf explants. Remarkably, the first variegated leaves arose in an R_4 population obtained after repeated selfing of the "57" dwarf type, whereas one would expect the R_2 generation to express recessive characters. Apparently, the *sulf* mutation arose spontaneously but it cannot be excluded that the unknown mechanism responsible for the instability of "57" dwarf also induces the instability at the *sulf* locus. No other *sulfurea* genotypes have been recovered among several generations of the unstable "57" dwarf type of which at least a few tens were analyzed.

The newly-isolated *sulfurea* mutation possesses the same two singular features of the original *sulfurea* allele in that it gives rise to pura seedlings and is paramutagenic. The fact that wild-type *sulf*$^{+}$ alleles changed to *variegata* in response to the SC148 allele in (*sulf*$^{+}$/*sulf*^{vag}) hybrids with a variegated phenotype was confirmed by showing the presence of the long satellite typical for the wild-type allele.

The percentage of variegated plants differed significantly among the hybrids derived from crosses between SC148 and various tomato lines. The level of paramutagenicity of the new *sulfurea* allele was much higher than the original *sulf*^{vag} allele, the maximum being 50% for the SC148 allele as compared to 12% for the original *sulf* allele. It is important to note, however, that Hagemann used different tomato genotypes in his crosses. Another remarkable difference was the appearance of *pura* plants among the F_1 hybrids. In Hagemann's material, the change from *sulf*$^+$ to *sulf*^{vag} and to *sulf*^{pura} occurred stepwise and unidirectionally. If this also applies to our mutant, then, in order to form F_1 hybrids (*sulf*$^+$/*sulf*^{vag}) with a pura phenotype, the wild-type locus must have mutated to *variegata* and subsequently to *pura* between fertilization and germination. In the same time span the *sulf*^{vag} allele (SC148) had mutated to *pura*. An alternative explanation would be that a *sulf*^{pura} allele, not a *sulf*^{vag} allele, was present in the F_1 heterozygote. If this pura allele could then alter the wild type *sulf*$^+$ allele directly into a pura allele only one alteration could account for the appearance of puras among the F_1. Whatever the case, the new *sulfurea* mutation seems to be active at an earlier stage in development as compared to the original *sulfurea* mutant, in which the paramutation process seems to start after seed germination (Hagemann and Berg 1978).

There has been much speculation about the molecular basis of paramutation. In *Antirrhinum*, the interaction of two different *Tam* elements inserted in the respective paramutagenic and paramutable *nivea* alleles was thought to be involved in the paramutagenic effect (Krebbers et al. 1987). In the case of the *R* locus of maize, the methylation of sites at the *R* allele was correlated with the paramutagenicity (Dooner et al. 1991). Since the *sulfurea* locus has not yet been cloned, molecular data are not available. In considering possible explanations for the instability at the *sulf* locus, two aspects of the *sulfurea* mutation need to be distinguished. Firstly, the variegated patterns in leaf color suggest that the inactivation of the *sulf* allele occurs in some cells but not in others. Secondly, its paramutagenic behaviour requires the imposition of the inactive state on the wild-type *sulf*$^+$ allele. These features resemble a special case of position effect variegation (PEV) at the *brown* locus in *Drosophila* (Henikoff and Dreesen 1989). When the *brown* locus is rearranged close to heterochromatin its expression becomes unstable giving rise to variegated patterns in eye color, just as with the variegated patterns of the *sulf* mutant. Furthermore, the unstable *brown* allele inactivates in trans the unrearranged wild-type allele, comparable to the in-trans inactivation of the wild-type *sulf*$^+$ allele. In case of the *brown* gene the variable inactivation is thought to be the result of the spreading of heterochromatin into the *brown* gene. Apparently, this heterochromatinization is then imposed on the wild-type *brown* allele. It is highly plausible that such a model also accounts for the instability at the *sulf* locus, as was proposed earlier by Hagemann (1969). In this context it is suggestive that the *sulf* locus maps close to, or possible within, the heterochromatic region of chromosome 2. Moreover, the *sulf* mutation is recovered after a treatment with X-rays that is known to cause chromosomal rearrangements.

There are additional suggestive similarities with PEV. In general the level of PEV can be influenced by the amount of heterochromatin present in the nucleus. This influence was also observed for the *sulf* mutation when crossed to the 2s-Iso line that carries an extra chromosome consisting of two completely heterochromatic arms of chromosome 2. As judged from the high percentage of mutants among this F_1 population it appeared that *sulf* instability is enhanced in response to an extra dosage of heterochromatin, a response that is very similar to that of the *light* gene of *Drosophila* (Devlin et al. 1990; Gatti and Pimpinelii 1992). This so called heterochromatic gene behaves opposite to the euchromatic genes in that it becomes unstable when positioned in euchromatin. The instability of the *light* gene is enhanced by adding extra heterochromatin in the form of Y chromosomes (Devlin et al. 1990; Henikoff 1990). Likewise, *sulf* may be a heterochromatic gene only correctly expressed in a heterochromatic environment. Final proof that a position effect is involved in the paramutagenicity of the *sulf* locus may be obtained by translocating the gene to a euchromatic environment and then studying its expression at the new site, as has been achieved for the *P* locus in *Oenothera* (Catcheside 1947).

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