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Evidence for a revertant at the *La* locus in regenerating hypocotyl segments in tomato

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Summary. Leafy and leafless phenotypes were regenerated in vitro from hypocotyl segments of leafless forms (reduced and modified) of the homozygous *lanceolate* (*La*) mutant in tomato. Segregation of progeny of leafy regenerates into homozygous mutant (*La La*), heterozygote (*La* La^+) and normal (La^+ La^+) indicates that cells forming the shoot apical meristems undergo a genetic reversion, and that the nutrient medium might be selecting for the heterozygote. Among the progeny of the regenerates is a true breeding, unlobed variant. Leaves of the variant are pinnately compound and the margins are entire. Opposite cotyledons followed in development by two simple leaves before the appearance of a pinnately compound leaf with an occasional lanceolate-shaped leaflet suggests that the unlobed variant is morphologically intermediate between *La* La^+ and La^+ La^+.

Key words: *Lycopersicon esculentum* L. – Reversion – Regeneration – Variant – *Lanceolate*

Introduction

The *La* gene in tomato is responsible for the development of a simple, lanceolate-shaped leaf which characterizes the heterozygote. When present in double dosage, the mutant allele shows a variable expressivity which is related to nutrient supply (Stettler and Alldredge 1967; Franco and Caruso 1985). The most progressed form of the homozygous mutant has numerous small leaves, weak apical dominance and sterile, tendril-like inflorescences. The least progressed form lacks leaves, cotyledons and shoot apex. The latter is a short-lived cylindrical structure which is equivalent to a hypocotyl. This unusual phenotype was designated reduced by Mathan and Jenkins (1962) and it becomes established early in embryogeny (Caruso 1968). The modified phenotype is also a homozygous mutant form which has a single, fleshy cotyledon with an occasional swelling at its base which is interpreted as an abortive bud.

Hypocotyl segments of reduced seedlings can form leafy shoots in vitro (Hangarter and Caruso 1982). This report describes the instability of the *La* gene during adventitious shoot formation from both modified and reduced phenotypes in vitro, as well as during regeneration from heterozygous *lanceolate* hypocotyl segments. The instability of the mutant allele in dividing cells of the cultured hypocotyl segment is expressed as an apparent reversion to the wild type allele.

Materials and methods

Seeds from greenhouse-grown tomato (*Lycopersicon esculentum* Mill.) of the isogenic line of *lanceolate* (LA 335) included homozygous mutant (*La La*), heterozygous *lanceolate* (*La* La^+) and homozygous normal (La^+ La^+) genotypes in the expected ratio of 1:2:1. Of the several different phenotypes shown by *La La*, only the leafless forms, reduced and modified, were used in our experiments. *Lanceolate* and normal tomato were also used as sources of hypocotyl explants.

Seeds were surface sterilized in 5% commercial Clorox for 5 min, followed by three rinses with sterile water, sown on moistened blotter paper in Petri dishes and incubated at 23 °C in the dark. Germinated seeds were transferred to test tubes (150×25 mm) containing 10 ml of either double strength Knop's macronutrients and 1 ml Nitsch's micronutrients (Nitsch 1951) or half-strength MS basal medium (Murashige and Skoog 1962). Both media contained 2% sucrose (w/v) and were solidified with 0.7% agar.

Hypocotyl segments, 5 mm in length, were excised from two-week old test-tube grown seedlings. The hypocotyl segments were placed horizontally on autoclaved MS medium containing 3 mg/l 6-benzylaminopurine and 1.5 mg/l indole-3-acetyl-L-phenylalanine (Research Organics, Cleveland). Cultures were maintained in incubators at 23 °C under cool white fluorescent lamps at 1100 lux in an 18-h photoperiod.

Adventitious shoots (RO) which were produced by the hypocotyl segments were transferred to MS medium containing 1 mg/l indole-3-butyric acid (IBA) for the purpose of producing roots, which occurred under the same environmental conditions as shoot induction. After approximately 30 days, rooted RO specimens were transplanted to soil and were grown to maturity in the greenhouse. The progeny (R1) of selfed RO were classified according to phenotype.

Results

Morphological changes in the cultured hypocotyl segments occurred between 8 and 30 days after transfer of the explants to the nutrient medium. In regenerating segments, the first sign of growth was cell division at both ends of the segment. Segments that formed many shoots usually produced a small amount of callus; those which formed few or no shoots produced an abundant callus. Furthermore, there was an inverse relationship between the capacity for regeneration and dosage of the *La* gene (Table 1).

Several vigorous shoots produced from segments of reduced and modified phenotypes had many simple leaves. An uncounted number of regenerates from the same genotype (*La La*) soon aborted after forming a few small leaves while others resembled the leafless forms from which they arose. Only the leafy RO shoots rooted well when they were cut away from the callus and transferred to the IBA-containing medium. When the rooted specimens were transferred to soil, they produced larger lanceolate leaves and had a stronger apical dominance than seen in *La* La^+ or *La La* which were grown from seed. These mature, leafy RO of homozygous mutant origin often showed features associated with high ethylene production, i.e. thick stems and dark green leaves with epinastic curvature. The leaves frequently revealed necrotic spots under conditions of high humidity. These features are not found in plants raised from seed. Tendril-like structures in place of flowers and flowers with shortened petals were also common. Such floral traits are also common in the progressed form of *La La* from seed. Regenerates from heterozygous *lanceolate* and homozygous normal explants did not show these aberrant features.

Fruits of *La La* regenerates were more elongated than those of heterozygous or normal origin, and although seed set was generally low, enough seed was collected to observe segregation in the R1 of selfed RO (Table 2).

In addition to the presence of the homozygous mutant, heterozygous *lanceolate* and homozygous normal in R1, there also appeared a variant with unlobed, pinnately compound leaves (Fig. 1). While the entire leaflets of the variant are frequently larger and broader than those of normal or *lanceolate,* an occasional leaflet is lanceolate in shape. Thus the variant plant displays a development which is intermediate between the heterozygote with simple, lanceolate leaf and normal tomato with compound, lobed leaves (Fig. 2). Furthermore, the variant seedling has opposite cotyledons (a normal trait), but forms two or three simple, unlobed leaves (a *lanceolate* trait) before producing its pinnately compound leaf with entire leaflets. Here too, the variant seedling shows a combination of features that suggest intermediacy in development (Fig. 3).

The variant has appeared in low numbers in the progeny of different RO from homozygous mutant as well as from heterozygous explants (Table 2). When the

Table 1. Frequency of regenerating cultured hypocotyl segments according to genotype. The number that regenerated (numerator) over the number of nonregenerated segments (denominator) gives a significant difference between observed and expected ($\chi^2 = 41.84$, 2 d.f.), using a 2×3 contingency table

	La La	*La* La^+	La^+ La^+
Observed	13/104	24/55	26/16
Expected			
Regenerating	30.9	20.9	11.1
Not	86	58.1	30.9

Table 2. Segregation of phenotypes in progeny of regenerates (RO) from cultured hypocotyl segments. The number of the unlobed variant, in combination with normal phenotype, comprises 25% of progeny (R1) from RO of *La La* and *La* La^+ origins

Genotype of hypocotyl segment	R1 seeds	Phenotype of R1 seedlings				
		Homozygous mutants[a]	Lanceolate	Normal	Unlobed variant	X^2
La La	826[b]	185	436	104	101	3.531
La La^+	351	90	173	69	19	0.112
La^+ La^+	970	0	0	970	0	

[a] Leafless and progressed counted
[b] 10 RO specimens from modified and 1 RO specimen from reduced gave 826 seeds

Fig. 1. Leaf of unlobed variant which is pinnately compound, with entire leaflets (×0.7)

Fig. 2. Leaf of *lanceolate* (*right*) and pinnately compound leaf with lobed leaflets of normal (*left*) (×0.7)

Fig. 3. Variant seedling with opposite cotyledons, two simple foliar leaves and unlobed compound leaves (×0.9)

numbers of the variant class are added to the class of normal, the combined totals constitute 25% of the progeny, giving a 1:2:1 ratio (Chi-square analyses). The variant breeds true, but a genetic determination of the unlobed locus remains to be done.

Regenerates of heterozygous *lanceolate* hypocotyl segments appeared, more or less, as their donor types, as already stated, but R1 of selfed RO from *lanceolate* explants also contained the unlobed variant (Table 2). Regenerates of homozygous normal origin appeared normal in every respect. Their progeny did not segregate and showed uniform, pinnately compound leaves with lobed leaflets (Table 2).

Discussion

There are several reports of mutation being responsible for genetic variation in plants arising from tissue culture (Evans and Sharp 1983), but to our knowledge, this is the first report of what appears to be a recurrent, genetic reversion. The swollen stems, strong apical dominance and poorly developed flowers of regenerates suggested early in the study that some genetic change in RO was possible. The segregation of progeny from selfed regenerates of *La La* origin confirms the genetic instability of the *La* locus. In addition, the presence of an unlobed variant in R1 from both heterozygous and homozygous mutant origins signals either a change in the mutant allele or in the control thereof.

Franco and Caruso (1985) observed a significantly greater number of leafless phenotypes in *La La* progeny from unfertilized parent plants. Thus the expression of the *La* gene in the homozygous condition had been shown to be affected by nutrient stress; however, the instability of the gene in connection with the reversion reported here is not well enough understood to ascribe to environmental conditions. It is possible that the nutrient medium is acting as a selective agent or sieve, a principle described by Evans et al. (1984). If so, the heterozygote ($La\ La^+$) in the present study might have a selective advantage which is expressed early in the development of shoot apical meristems of cultured hypocotyl segments. The appearance of abortive shoots which resemble homozygous mutant phenotypes would lend support to this hypothesis. Carlson et al. (1984) cite *Drosophila* as an example in which nonlethal mutations were superior, when heterozygous against a homozygous genetic background. More cases of heterosis may also be uncovered in plants regenerating from tissue culture, considering the increasing number of reports of mutational events.

Preliminary work shows that the unlobed variant breeds true. Further genetic analysis is required to determine the nature of the factor underlying this new form.

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