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# Inheritance of zingiberene in *Lycopersicon*

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**Abstract.** The inheritance of the sesquiterpene zingiberene was analyzed in segregating progeny of interspecific crosses among *Lycopersicon hirsutum* f. *hirsutum* Humb. and Bonpl. (*hir*), *L. esculentum* Mill. cv 'Nova' (*esc*), and *L. hirsutum* f. *glabratum* C.H. Mull (*gla*). The presence of zingiberene was inherited as a single dominant gene from *hir* in $F_2$ and BC progeny of *esc* × *hir*, and as a single recessive gene in $F_2$ and BC progeny of *gla* × *hir*. The segregation of *esc* × *hir*, *gla* × *hir*, and *esc* × *gla* progeny supported a single locus allelomorphic model in which the presence of zingiberene is controlled at a single locus, *Z*, where the allele from *hir* confers presence of zingiberene and is dominant to the allele from *esc* but recessive to that from *gla*. The presence of zingiberene in *esc* × *hir* progeny was not linked to the ability to set fruit or to several fruit characters, and progeny with zingiberene levels comparable to *hir* were recovered.

**Key words:** Tomato – *Lycopersicon hirsutum* – Sesquiterpene – Zingiberene – Host-plant resistance

## Introduction

The sesquiterpene zingiberene accumulates in large amounts in the Type VI trichomes of *Lycopersicon hirsutum* f. *hirsutum* Humb. and Bonpl. PI 126445 (*hir*) (Carter et al. 1989a) and is associated with the resistance of *hir* to the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (CPB) (Carter et al. 1989b). The related sub-species *L. hirsutum* f. *glabratum* C.H. Mull (*gla*) lacks zingiberene, but is resistant to CPB due to the compound 2-tridecanone (Kennedy and Sorenson 1985). However, in *gla* × *hir* progeny segregating for both zingiberene and 2-tridecanone, CPB resistance has been primarily associated with the presence of zingiberene, which was conferred by one or two recessive genes (Carter et al. 1989b).

Though the inheritance of zingiberene appeared to be relatively simple in crosses between the sub-species *hir* and *gla*, its usefulness as a source of host-plant resistance depends upon the ability to transfer zingiberene accumulation into cultivated tomato, preferably without introducing linkages to undesirable traits. Our objectives in the investigation presented here were (1) to determine with greater precision the number of genes controlling zingiberene accumulation in *gla* × *hir*, and (2) to determine the mode of inheritance of zingiberene in the interspecific cross of *hir* to the cultivated tomato *L. esculentum*.

## Materials and methods

*Plant materials and crosses*

Seed of *Lycopersicon hirsutum* f. *hirsutum* Humb. and Bonpl. PI 126445 (*hir*) and *L. hirsutum* f. *glabratum* C.H. Mull PI 134417 (*gla*) were obtained from the North Central Regional Plant Introduction Station, Ames, Iowa, and selected individuals were propagated by cuttings. *Lycopersicon esculentum* Mill. cv 'Nova' (*esc*) seeds were obtained from Stokes Seeds, Buffalo, New York.

Crosses were made using *esc* or *gla* as female parents and *hir* as the male parent. The reciprocal crosses were not possible due to unilateral incompatibility associated with *hir* (Martin 1963). Parent and $F_1$ plants of *esc* × *hir* and *gla* × *hir* were grown in the greenhouse at 20°–27 °C and under a 14 h light/10 h dark regime.

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$F_2$ families were produced from intercrosses among $F_1$ siblings, which inherited the self-incompatibility of *hir* (McGuire and Rick 1954). Four BC families, *esc* × (*esc* × *hir*), (*esc* × *hir*) × *hir*, *gla* × (*gla* × *hir*), and (*gla* × *hir*) × *hir*, were produced in the fall of 1988.

After germination in the greenhouse in the spring of 1989, the six families and seedlings or rooted cuttings of the parents were transplanted to the field at New Brunswick NJ on June 1. There were 208 plants in each $F_2$ family, 80 plants in each backcross family, and 10 plants of each parent. Plants were completely randomized, with 5 feet between plants in each row and 6 feet between rows. Plants were hand cultivated 3 times during the growing season and irrigated as needed. For analysis of zingiberene content, the middle leaflet of the third leaf from the apex was collected from each plant in July 1989. For analysis of linkage of zingiberene inheritance with fruit set and fruit characters, zingiberene assays were repeated in August 1989.

To examine the possibility that regulatory factors suppressing zingiberene production were present in *gla*, *esc* was crossed to *gla*, and $F_1$ seeds were produced in the fall of 1989. The $F_1$ progeny were selfed and backcrossed to *esc* to produce $F_2$ and BC families. Seeds were planted in the greenhouse in January 1990 and 100 plants of each family were analyzed at 90 days for zingiberene content.

### *Identification and analysis of zingiberene*

Zingiberene contents of the parents and progeny were determined by GC analysis of hexane extracts as previously described (Carter et al. 1989b). Identification and quantification of zingiberene in the extracts were made by comparison to the peak retention time and peak area of zingiberene in *hir* leaflets. For the backcross family of *gla* × (*gla* × *hir*), GC-MS was used as previously described (Carter et al. 1989b) to distinguish between zingiberene and 2-tridecanone, which have similar retention times under some chromatographic conditions (Snyder et al. 1987).

### *Inheritance of zingiberene and fruit characters*

Zingiberene levels of the progeny were converted to relative zingiberene content as a percentage of the zingiberene level in *hir* leaflets collected at the same time and location. Ability to set fruit and three fruit characters, shape (long vs. round), presence of a beak at the stylar end, and presence of a green stripe, were determined by visual inspection. Models of inheritance for zingiberene content, fruit set and fruit characters were analyzed by chi-square.

## Results

### *Inheritance of zingiberene*

In segregating progeny of *gla* × *hir*, the presence of zingiberene was conferred by a single recessive gene (Table 1), as previously suggested (Carter et al. 1989b). Of the 39 (*gla* × *hir*) × *hir* BC progeny with zingiberene, 29 (74%) had zingiberene levels equal to or greater than that of *hir* leaflets collected at the same time and location. Of the 48 *gla* × *hir* $F_2$ progeny with zingiberene, 24 (50%) had zingiberene levels equivalent to that of *hir*.

In *esc* × *hir* progeny, the presence of zingiberene was inherited as a single dominant gene in both the $F_2$ and BC populations (Table 1). Among *esc* × *hir* $F_2$s in which zingiberene was present, the level of zingiberene ranged from 1% to 100% of the zingiberene level found in *hir* leaflets, which averaged 250 µg per leaflet. Of the $F_2$s with zingiberene, the majority (120 individuals) had 50% or less than the level of zingiberene present in *hir*, and 27 individuals had 50–100% of the *hir* zingiberene level. The data do not support a simple model of codominance for zingiberene level, but suggest that additional genes may modify the level of zingiberene production.

To examine the possibility that *gla* contained alleles at another locus that suppressed zingiberene production, 100 *esc* × *gla* $F_2$s and 100 *esc* × (*esc* × *gla*) BC

**Table 1.** Segregation for the presence or absence of zingiberene in $F_2$ and BC populations of *L. hirsutum* f. *glabratum* (*gla*) × *L. hirsutum* f. *hirsutum* (*hir*), *L. esculentum* cv Nova (*esc*) × *L. hirsutum* f. *hirsutum* (*hir*), and *L. esculentum* cv 'Nova' (*esc*) × *L. hirsutum* f. *glabratum* (*gla*)

| Population | +Z:−Z[a] | Expected ratio[b] | $\chi^2$ | $P$ |
|---|---|---|---|---|
| *gla* × *hir* | | | | |
| $F_2$ | 48:155 | 1:3 | 0.13 | $0.70 < P < 0.90$ |
| BC to *gla* | 0:24 | 0:1 | 0.01 | $0.90 < P < 0.95$ |
| BC to *hir* | 39:40 | 1:1 | 0.01 | $0.90 < P < 0.95$ |
| *esc* × *hir* | | | | |
| $F_2$ | 145:50 | 3:1 | 0.02 | $0.50 < P < 0.70$ |
| BC to *esc* | 32:36 | 1:1 | 0.24 | $0.50 < P < 0.70$ |
| BC to *hir* | 70:0 | 1:0 | 0.004 | $0.95 < P$ |
| *esc* × *gla* | | | | |
| $F_2$ | 0:100 | 0:1 | 0.002 | $0.95 < P$ |
| BC to *esc* | 0:100 | 0:1 | 0.002 | $0.95 < P$ |

[a] Number of plants with zingiberene (+Z) versus the number lacking zingiberene (−Z)

[b] Ratios expected if presence of zingiberene is conditioned by a single gene *Z* from *hir* that is expressed as a recessive in the cross to *gla* and as a dominant in the cross to *esc*

**Table 2.** Expected ratios for two models explaining the mode of action at the *Z* locus in segregating progeny of *gla* × *hir* and *esc* × *hir*

| A) *gla* × *hir* Generation | Model I ($Z^2 > Z^1 > Z^3$)[a] | Model II ($Y\_ > Z\_$)[b] |
|---|---|---|
| Parents | *gla* × *hir*<br>$Z^2Z^2$ $Z^1Z^1$<br>(−Z) (+Z) | *gla* × *hir*<br>*ZZYY* *ZZyy*<br>(−Z) (+Z) |
| $F_1$ | $Z^1Z^2$<br>(−Z) | *ZzYy*<br>(−Z) |
| $F_2$ | 3 $Z^2\_$:1 $Z^1Z^1$<br>(−Z) (+Z) | 3 *ZZY_*:1 *ZZyy*<br>(−Z) (+Z) |
| BC to *gla* | 1 $Z^2Z^2$:1 $Z^1Z^2$<br>(all −Z) | 1 *ZZYY*:1 *ZZYy*<br>(all −Z) |
| BC to *hir* | 1 $Z^1Z^2$:1 $Z^1Z^1$<br>(−Z) (+Z) | 1 *ZZYy*:1 *ZZyy*<br>(−Z) (+Z) |
| **B) *esc* × *hir* Generation** | **Model I ($Z^2 > Z^1 > Z^3$)[a]** | **Model II ($Y\_ > Z\_$)[b]** |
| Parents | *esc* × *hir*<br>$Z^3Z^3$ $Z^1Z^1$<br>(−Z) (+Z) | *esc* × *hir*<br>*zzyy* *ZZyy*<br>(−Z) (+Z) |
| $F_1$ | $Z^1Z^3$<br>(+Z) | *Zzyy*<br>(+Z) |
| $F_2$ | 3 $Z^1\_$:1 $Z^3Z^3$<br>(+Z) (−Z) | 3 *Z_yy*:1 *zzyy*<br>(+Z) (−Z) |
| BC to *esc* | 1 $Z^1Z^3$:1 $Z^3Z^3$<br>(+Z) (−Z) | 1 *Zzyy*:1 *zzyy*<br>(+Z) (−Z) |
| BC to *hir* | 1 $Z^1Z^1$:1 $Z^1Z^3$<br>(all +Z) | 1 *ZZyy*:1 *Zzyy*<br>(all +Z) |

[a] Model I describes *Z* as an allelomorphic locus, where $Z^1$ confers presence of zingiberene and $Z^2$ and $Z^3$ confer absence of zingiberene, with $Z^1$ dominant to $Z^3$ but recessive to $Z^2$ ($Z^2 > Z^1 > Z^3$)

[b] Model II describes *Z* as a dominant/recessive locus with epistatic interactions due to suppression of *Z* by a second locus, *Y* (*Y_* > *Z_*)

progeny also were examined for zingiberene content. None of these 200 individuals contained zingiberene (Table 2).

Of the six families examined, only the BC *esc* × (*esc* × *hir*) produced fruit, and there was no linkage between fruit set and zingiberene production in this family (Fig. 1). Of the 68 plants in this family, 42 produced fruit, which were collected and scored for three fruit characters: presence of a beak at the stylar end, derived from 'Nova'; presence of a green stripe, derived from *hir*; and long versus round fruit shape, with the long shape derived from 'Nova' and the round one from *hir*. The presence of a beak on the fruit was conditioned by a single recessive gene (Fig. 2A), which is in agreement with the description of *bk* by Young and MacArthur (1947) and Rick and Butler (1956). The appearance of a green stripe was also inherited as a monorecessive (Fig. 2B), corresponding to *gs* inheritance described by Larson and Pollack (1951). Fruit shape was inherited as a single recessive gene (Fig. 2C), as expected for the *el* locus described by Butler (1952). There was no linkage between zingiberene production and any of the three fruit characters (Fig. 3).

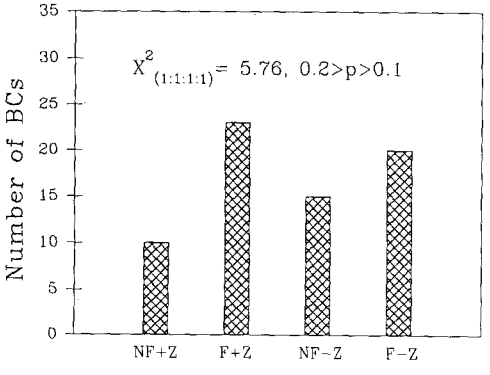


**Fig. 1.** Segregation of zingiberene in combination with ability to set fruit. Fruit set (*F*) or no fruit set (*NF*), zingiberene present (+*Z*) or absent (−*Z*)

## Discussion

In both *gla* × *hir* and *esc* × *hir*, the presence of zingiberene was conferred by a single gene, here designated *Z*. However, in $F_2$ and BC populations of *gla* × *hir*, *Z* was inherited as a single recessive gene,

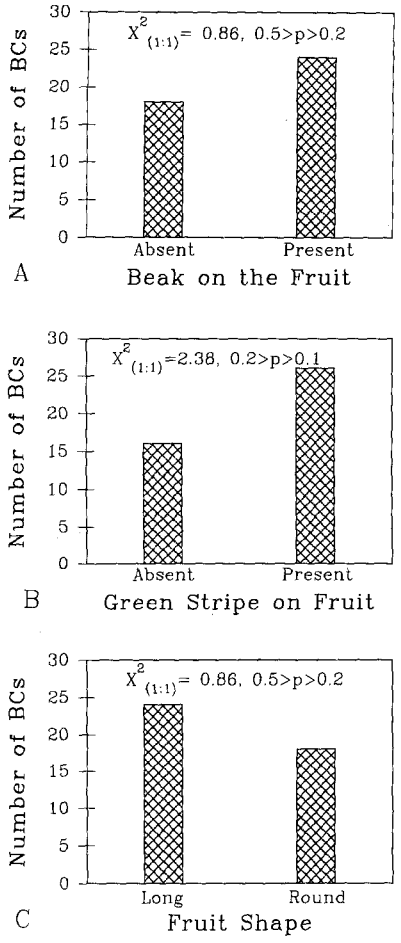


**Fig. 2A–C.** Segregation for fruit characters in the BC population of *Lycopersicon esculentum* cv 'Nova' × *Lycopersicon hirsutum* f. *hirsutum*. **A** Beaked fruit (*bk*), **B** green stripe (*gs*), **C** long or round fruit shape (*el*)

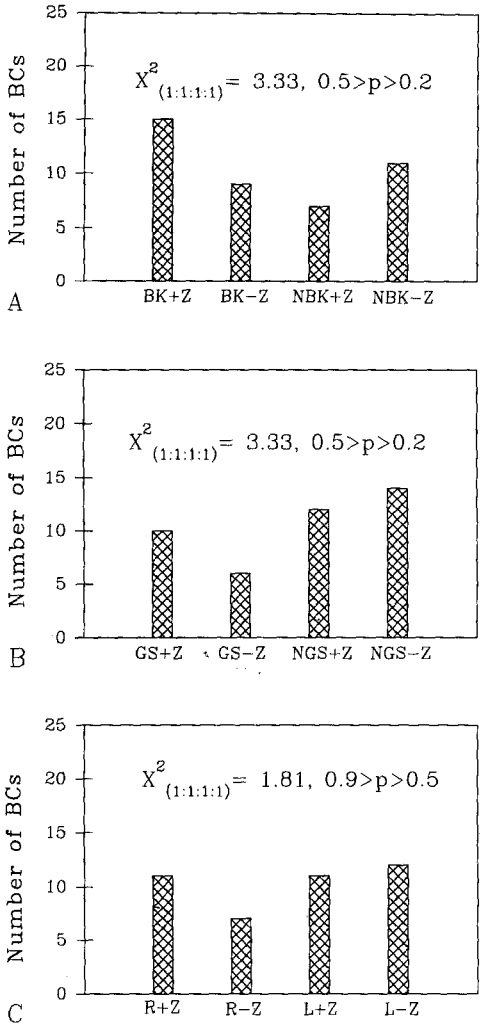


**Fig. 3A–C.** Segregation of zingiberene in combination with each of three fruit characters. **A** Fruit with (*BK*) or without a beak (*NBK*), and zingiberene present (+*Z*) or absent (−*Z*); **B** fruit with (*GS*) or without (*NGS*) a green stripe, and zingiberene present (+*Z*) or absent (−*Z*); **C** long (*L*) or round (*R*) fruit shape, and zingiberene present (+*Z*) or absent (−*Z*)

while in the $F_2$ and BC populations of *esc* × *hir*, *Z* was inherited as a single dominant gene.

Two models were investigated to explain the observed ratios of *Z* as dominant in one background and recessive in the other. Model I: The *Z* locus may be allelomorphic, where $Z^1$ from *hir* confers presence of zingiberene and $Z^2$ from *gla* and $Z^3$ from *esc* confer absence of zingiberene, with $Z^2$ dominant to $Z^1$, and $Z^1$ dominant to $Z^3$ ($Z^2 > Z^1 > Z^3$) (Table 2A). Model II: The expression of *Z* may by affected by epistatic interactions between *Z* and a hypothetical locus *Y*, where a dominant allele at *Y* in *gla* suppresses *Z* activity, and both *hir* and *esc* have recessive *Y* alleles allowing *Z* expression (Table 2B). In this model, *hir* would be *ZZ*, but *gla* could be either *ZZ* or *zz*. If *gla* is *ZZ*, the expected *gla* × *hir* $F_2$ ratio is 3:1 for absence: presence of zingiberene; if *gla* is *zz*, a ratio of 13:3 for absence:presence would be expected. As indicated in Table 1, $\chi^2_{3:1}$ for *gla* × *hir* was 0.13 ($0.70 < P < 0.90$), whereas $\chi^2_{13:3} = 1.16$ ($0.20 < P < 0.30$), i.e., the fit is better with the 3:1 model where both *hir* and *gla* are *ZZ*. Thus, *gla* is presented in Model 2 as *ZZYY*.

Both models predict the ratios observed in the crosses of *esc* × *hir* and *gla* × *hir*. However, if Model II is correct, i.e., if both *hir* and *gla* possess dominant alleles at *Z* but *gla* also has a dominant allele at *Y* that suppresses *Z* and *esc* has recessive alleles at both *Z* and *Y*, then one-fourth of recombinant *esc* × *gla* $F_2$ and *esc* × (*esc* × *gla*) BC progeny would possess a dominant *Z* gene as well as homozygous recessive alleles at the *Y* locus from *esc*, and thus produce zingiberene. If, on the other hand, Model I is correct, none of the *gla* × *esc* $F_2$ or BC progeny should have zingiberene. The ratios predicted by Models I and II for the cross of *esc* × *gla* are presented in Table 3.

**Table 3.** Expected ratios for two models explaining the mode of action at the *Z* locus in segregating progeny of *esc* × *gla*

| Generation | Model I ($Z^2 > Z^1 > Z^3$)[a] | Model II ($Y\_ > Z\_$)[b] |
|---|---|---|
| Parents | *esc* × *gla*<br>$Z^3Z^3$ $Z^2Z^2$<br>(−Z) (−Z) | *esc* × *gla*<br>*zzyy* *ZZYY*<br>(−Z) (−Z) |
| $F_1$ | $Z^2Z^3$<br>(−Z) | *ZzYy*<br>(−Z) |
| $F_2$ | 1 $Z^2Z^2$:2 $Z^2Z^3$:1 $Z^3Z^3$<br>(all −Z) | 9 *Z_Y_*:3 *zzYy*:3 *Z_yy*:1 *zzyy*<br>(−Z) (−Z) (+Z) (−Z) |
| BC to *esc* | 1 $Z^2Z^3$:1 $Z^3Z^3$<br>(all −Z) | 1 *ZzYy*:1 *Zzyy*:1 *zz Yy*:1 *zzyy*<br>(−Z) (+Z) (−Z) (−Z) |
| BC to *gla* | 1 $Z^2Z^2$:1 $Z^2Z^3$<br>(all −Z) | *Z_Y_*<br>(all −Z) |

[a] Model I describes *Z* as an allelomorphic locus, where $Z^1$ confers presence of zingiberene and $Z^2$ and $Z^3$ confer absence of zingiberene, with $Z^1$ dominant to $Z^3$ but recessive to $Z^2$ ($Z^2 > Z^1 > Z^3$)

[b] Model II describes *Z* as a simple dominant/recessive locus with epistatic interactions due to suppression of *Z* by a second locus, *Y* ($Y\_ > Z\_$)

To distinguish between these two models, 100 BC and 100 $F_2$ progeny of *esc* × *gla* were examined for zingiberene content. None of the $F_2$ or BC progeny of *esc* × *gla* contained zingiberene (Table 1), leading us to reject Model II. Although other models may also explain the mode of action of *Z*, the observed ratios support a single locus model, possibly allelomorphic, where alleles from *hir*, *gla*, and *esc* may be designated $Z^1$, $Z^2$, and $Z^3$, respectively, and display a dominance relationship of $Z^2 > Z^1 > Z^3$.

The presence of zingiberene was inherited as a single dominant gene in crosses to cultivated tomato, and progeny with zingiberene levels comparable to those of *hir* were recovered. The presence of zingiberene was not linked to the ability of *esc* × (*esc* × *hir*) BC progeny to set fruit or to several undesirable fruit characters. The simple pattern of inheritance, the lack of linkage to fruit set, and the ability to recover recombinants with high zingiberene levels suggest that insect resistance associated with zingiberene may be easily transferred to cultivated tomato in a breeding program.

One caveat to this proposal: we have observed that zingiberene levels are lower in the summer than in the winter, and recent analyses indicate significant temperature and photoperiod effects on zingiberene accumulation (Gianfagna et al. 1992). Larval mortality on intact leaves of *hir* and *esc* × *hir* $F_2$s with high zingiberene levels, while significantly greater than mortality on *esc* and $F_2$s lacking zingiberene (Rahimi 1990), was also less in summer trials than previously observed for *hir* grown in the fall or winter. Thus, the potential usefulness of zingiberene as a source of host-plant resistance to insects must be mitigated by its unfortunate tendency to be most abundant when least needed.

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