Molecular and Ultrastructural Analysis of a Nonchromosomal Variegated Mutant

Tomato Mitochondrial Mutants That Cause Abnormal Leaf Development

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Mutants were recovered in a population of cybrids formed following protoplast fusion between tomato (Lycopersicon esculentum Mill.) cv UC82 and Lycopersicon pennellii Corr. The cybrids were identified as individuals with recombinant cytoplasmic genomes but only tomato nuclear genomes. The mutants were identified based on two features, a variegated sectoring of light and dark green regions on their leaves, stems, and fruit, and reduced growth in the field. The mutants produced 50% of the shoot fresh weight and 20% of the fruit fresh weight of the parental type, UC82. The variegated sectoring was maternally inherited. The chloroplast genome in the mutants was indistinguishable from the chloroplast genome in UC82, when distribution of restriction endonuclease sites was used as an assay. The mitochondrial genome in the mutants, however, was recombinant, containing genes from UC82 and L. pennellii. Light microscopic analysis of the leaves of the mutants demonstrated an absence of the palisade layer in the variegated sectors. Electron microscopic analysis of these same regions demonstrated an absence of normal inner membranes in the mitochondria of these cells.

Plant growth and development are the result of coordinated expression of genes located primarily in the nucleus but also of genes located in the chloroplast and mitochondrion. The characterization of developmental mutants is a powerful approach to understanding the genetic control of growth and development. A common mitochondrial mutation is CMS (Grun, 1976). This phenotype is found in many genera, although the mutation in the mitochondrial genome differs among the individual male-sterile cytoplasmic lines (Levings, 1994). In addition to an effect on pollen
development, a role for the mitochondrial genome in vegetative growth and development is suggested as additional plant mitochondrial mutants have been identified. The best characterized of these are the NCS mutants in corn (Newton et al., 1990; Hunt and Newton, 1991; Roussell et al., 1991; Gu et al., 1993). A similar type of mutant was also described in tobacco (Bonnett et al., 1993). Leaf variegation in Arabidopsis thaliana has also been associated with alterations in mitochondrial genome organization (Martinez-Zapater et al., 1992).

To study the role of the mitochondrial genome in plant growth and development, alloplasmically substituted lines can be developed sexually or asexually. Sexually derived alloplasmically substituted lines of tomato are rare and difficult to generate because of strong unilateral incongruity in the genus (Hogenboom, 1979). Two examples of alloplasmic substitution have been reported in Lycopersicon. CMS was generated in Lycopersicon pennellii plants carrying cytoplasm from cultivated tomato (Anderson, 1964). In the reverse combination, tomato plants carrying L. pennellii cytoplasm, no differences in phenotype were detected with the unsubstituted maternal parent (Mutschler, 1990). In sexually derived alloplasmic lines, the chloroplast and mitochondria are both substituted and the individual organellar effects on growth and development cannot be determined.

In plants amenable to protoplast manipulations, alterations in organellar genomes can be generated following protoplast fusions, and chloroplast and mitochondrial effects can be separated (Galun and Aviv, 1983). Tomato is a plant amenable to protoplast fusion techniques, and we as well as others have regenerated a variety of symmetric and asymmetric fusion products following interspecific and intergeneric fusions (Wolters et al., 1994). In most of these cases, although there were alterations in the organization of the mitochondrial genome, no effects on plant growth and development could be attributed specifically to these

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Abbreviations: CMS, cytoplasmic male sterility; NCS, nonchromosomal stripe; NCV, nonchromosomal variegated; Rx, primary regenerant; Rx, progeny of Rx, selfed; RFLP, restriction fragment length polymorphism.
alterations in the mitochondrial genome. In one case, CMS tomatoes have been produced following the regeneration of tomato cybrids with portions of the mitochondrial genome of *Solanum acaule* (Melchers et al., 1992). We have constructed tomato cybrids that have tomato nuclear and chloroplast genomes but mitochondrial genomes that are recombinant between tomato (*Lycopersicon esculentum*) and *L. pennellii* (Bonnema et al., 1991). Several individuals in this cybrid population have an abnormal leaf development and reduced vigor. In this report we document the alteration in plant development, demonstrate maternal inheritance of the phenotype, describe at the ultrastructural level the effects of this mitochondrial mutation on leaf development, and describe the alterations in the mitochondrial genome associated with the phenotype. We propose to name this mutant NCV after the convention of NCS for maize (Newton and Coe, 1986).

### MATERIALS AND METHODS

#### Plant Materials

Cybrids were produced following protoplast fusion between cultivated tomato (*Lycopersicon esculentum*, Mill.) cv UC82 and *Lycopersicon pennellii*, Corr., LA716 (Bonnema, 1990; Bonnema et al., 1991, 1992). The cybrids were classified into one of two groups: group I contained plants with small, deformed, variegated leaves and greater than 50% *L. pennellii* mtDNA (plant Nos. 81, 100A, 100B, 100C, 100D, 100E, 121, and 122), and group II contained plants with normal leaves and less than 10% *L. pennellii* mtDNA (plant Nos. 92A, 92B, and 16A). The noncybrid regenerants were individuals that were recovered following protoplast fusion but scored as cultivated tomato at all of the nuclear and organellar loci tested. The noncybrid regenerants were also split into two groups: group III contained plants with an abnormal phenotype (plant Nos. 65D, 126A, 163A, 187, and 233A), and group IV was plants with a normal phenotype (plant Nos. 14, 63A, 64, 68, and 69A).

Selfed seed were obtained from the R₃. All of the plant materials described in this report were grown from seed. A field evaluation of growth was performed using the R₁ generation; the molecular analyses, ultrastructural analyses, and the reciprocal crosses were performed using more advanced selfed generations, R₂ through R₄ generations.

#### Field Experiment

Seeds were germinated in flats in a vermiculite:sand mixture in a growth chamber at 25°C. Following germination the seedlings were maintained in the greenhouse for approximately 5 weeks, fertilized with Miracle Gro (Stern, Port Washington, NY), and watered with Benomyl (Dexol, Torrance, CA) weekly. Six weeks after sowing, the plants were transplanted to the field at Leyendecker Plant Science Research Center, southwest of Las Cruces, NM. Plants were spaced at 60-cm intervals with 1 m between rows. The field was furrow irrigated and no fungicides, insecticides, or herbicides were used. A complete randomized block design with four blocks was used. Each block contained seven R₁ individuals from each of the 19 R₃s (treatments).

Within each block, two plots of seven plants were alternated with one UC82 control plant. After 22 weeks, the red fruit were hand harvested and weighed; at week 25 all remaining fruit were hand harvested and weighed, and at this time the shoot was harvested and the fresh weight was determined. The combined sum of the fruit harvested at week 22 and week 25 was reported. All statistical analyses were done using analysis of variance, with mean separation by the LSD test for variables with significant *F* tests only.

#### Chl Determination

Total Chl, Chl *a*, and Chl *b* levels were determined in acetone extracts of leaves as described by Arnon (1949). Equivalent-sized light-green or dark-green sectors were weighed and extracted to characterize the NCV mutants.

#### DNA Extraction and Southern Analysis

Total DNA, ctDNA, and mtDNA were isolated from fresh leaf tissue as described by Doyle and Doyle (1989), Saltz and Beckmann (1981), and Hanson et al. (1986), respectively. The methods described by Bonnema et al. (1991) were used for Southern hybridization. Oligonucleotide-labeled probes were prepared from gel-purified inserts of recombinant clones carrying the mitochondrial genes: *rrn26* (Stern et al., 1982), *rrn18* and *rrn5* (Chao et al., 1984), *nadI* (Chapdelaine and Bonen, 1991), *atpA* (Braun and Levings, 1985), *atp6* (Dewey et al., 1985), *atp9* (Young et al., 1986), *coxI* (Bon et al., 1987), *coxII* (Fox and Leaver, 1981), and *cob* (Boer et al., 1985). To identify a species-specific RFLP in ctDNA, oligonucleotide-labeled probes were prepared from gel-purified inserts of a recombinant clone carrying a 27-kb *SalI* fragment from tomato ctDNA (Bonnema et al., 1991).

#### Microscopy

Viable pollen was determined after staining with Alexander’s stain (Alexander, 1969). For microscopic analyses, leaf tissue of greenhouse-grown plants was collected directly into 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.2. The samples were postfixed in 0.1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Spurr’s resin. Semithin sections were cut, stained with 0.1% toluidine blue, examined, and photographed with a Leitz Ortholux microscope. Ultrathin sections of the areas of interest were prepared, stained with uranyl acetate and lead citrate, and then examined with a Hitachi (Tokyo, Japan) H7000 transmission electron microscope.

### RESULTS

#### Description and Inheritance of the NCV Phenotype

Tomato cybrids were generated following protoplast fusion between cultivated tomato, UC82, and irradiated protoplasts isolated from *L. pennellii* (Bonnema et al., 1991).
Cybrids are defined, in this case, as regenerants of protoplast fusion events with recombinant cytoplasms and diploid tomato nuclei. Several of the cybrids (group I) had smaller, variegated leaves and grew poorly. Commonly, R0s may have an abnormal phenotype as a result of the tissue-culture conditions. Analysis of the progeny of the R0s distinguishes heritable abnormalities from tissue-culture-induced abnormalities. A field experiment was designed to determine whether the reduction in vigor observed in the R0 population of group-I cybrids were heritable. Selfed seed were collected from the R0 generation of 19 R0s: 6 group-I cybrids, 3 group-II cybrids, 5 group-III noncybrids, and 5 group-IV noncybrids. The seed germination rate ranged from 70 to 92% among all four groups. All of the seedlings were similar in vigor. All of the seedlings of the group-I cybrids had variegated leaves (Fig. 1B). None of the seedlings in the other groups had variegated leaves. As soon as the plants were established in the field, differences in vigor became obvious as well (Fig. 1A). Throughout the season, the group-I cybrids produced 3 times less fruit than any other group and 6 times less fruit than the protoplast fusion parent, UC82 (Table I). The variegation pattern observed on the leaves of group-I cybrids was also observed on the fruit, in this case dark-green spots on a light-green fruit (data not shown). The group-I cybrids also had the lowest shoot mass, showing half the growth of the parental cultivar (Table I).

The field experiment demonstrated that the reduction in vigor and the leaf variegation in the group-I cybrids was heritable. Furthermore, there was little to no segregation of the fruit yield or shoot yield among the progeny of a given group (data not shown) and no segregation for leaf variegation. This pattern of inheritance suggested that the phenotype was inherited in a non-Mendelian manner. To test this directly, reciprocal crosses were performed between UC82 and two representative cybrids, 100C, a member of group I, and 92A, a member of group II. The progeny were scored after germination in the greenhouse for one of the elements of the mutant phenotype, variegated leaves (Table II). All of the progeny with 100C cytoplasm displayed the variegated leaf pattern, whereas none of the progeny of any of the other crosses displayed this phenotype. This pattern of inheritance is indicative of a cytoplasmically controlled trait. We propose to name this phenotype NCV.

All of the progeny of NCV mutants had a similar degree of leaf variegation. Although it was visible on seedlings, it became progressively worse as the plants matured. The light-green color could have predominated on all of the growth from a lateral meristem. Associated with the variegation was an incomplete expansion of the leaflet blade. This is visible to a moderate degree in Figure 1B. Those leaflets with light-green sectors were not as expanded as

| Table I. The variation in the means of fruit and plant fresh weights among the different progeny groups |
| Season-long fruit weight (g) and season-long shoot weight (g) are compared among group-I, mutant cybrids (100A, 100B, 100C, 100D, 100E, and 121); group-II, nonmutant cybrids (92A, 92B, and 16A); group-III, noncybrid regenerants with abnormal morphology (65D, 126A, 163A, 187, and 233A); and group-IV, noncybrid regenerants with normal morphology (14, 63A, 64, 68, and 69A). Values represent the means of the number of individuals shown in parentheses. Mean separation was by the LSD (0.05) test for variables with significant F values only; values followed by different letters are significantly different. |

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Fruit Wt</th>
<th>Shoot Fresh Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (168)</td>
<td>1188 A</td>
<td>1119 A</td>
</tr>
<tr>
<td>IV (140)</td>
<td>3117 B</td>
<td>1370 B</td>
</tr>
<tr>
<td>II (84)</td>
<td>4181 C</td>
<td>1522 BC</td>
</tr>
<tr>
<td>III (140)</td>
<td>4452 C</td>
<td>1686 C</td>
</tr>
<tr>
<td>UC82 (72)</td>
<td>6412 D</td>
<td>2253 D</td>
</tr>
</tbody>
</table>

Table II. Inheritance of leaf variegation in progeny from reciprocal crosses |

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of Seeds Planted</th>
<th>No. of Plants</th>
<th>No. of Variegated</th>
<th>No. of Nonvariegated</th>
</tr>
</thead>
<tbody>
<tr>
<td>100C × UC82</td>
<td>30</td>
<td>23</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>UC82 × 100C</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>92A × UC82</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>UC82 × 92A</td>
<td>30</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>
the surrounding dark-green regions. This deformation in leaflet blade expansion also became more pronounced as the plants matured. Ultimately, most of the leaves on the plants were deformed.

The percentage of viable pollen was determined in the plants described in Table II. Pollen viability in UC82, UC82 \( \times 100C \), and \( 100C \times UC82 \) was 97, 93, and 80\%, respectively. Unlike plants with UC82 cytoplasm, there was quite a bit of plant-to-plant variability among the plants with \( 100C \) cytoplasm; pollen viability ranged from 65 to 94\%. There was no correlation between the degree of pollen viability and the extent of light-green sectoring on the leaves of the individual plants. There was high fertility and no leaf color variegation in plants with the wild-type cytoplasm.

**Microscopic Analysis**

In tobacco, a variegated leaf pattern was observed in a cybrid plant (Bonnett et al., 1993). Microscopic analysis of the variegated sectors revealed a replacement of the palisade layer with spongy mesophyll in the light-green sectors. To determine whether the variegation pattern in the tomato cybrids had a similar cellular basis, sections of light- and dark-green sectors of leaves from cybrid \( 100C \) were examined. Photomicrographs of these regions are presented in Figure 2. The cells immediately below the epidermal cell layer in the light-green sectors of cybrid \( 100C \) did not have the characteristic organization of a palisade cell layer (Fig. 2C). Instead, cells in this layer were organized in the manner of the spongy mesophyll layer. The dark-green sectors of the same leaf had the cellular organization found in wild type (Fig. 2, A and B).

The subcellular structures in the cells of the light-green and dark-green sectors of leaves from cybrid \( 100C \) as well as the wild-type plant UC82 were examined by EM. Electron micrographs of the cells in the palisade layer of UC82 and the dark-green and light-green sectors of cybrid \( 100C \) are shown in Figure 3, A, B, and C, respectively. The subcellular organization of UC82 leaves and the dark-green sectors of cybrid \( 100C \) were indistinguishable (Fig. 3, A and B). The mitochondria and chloroplasts in all of the fields examined from these two preparations appeared similar with respect to abundance of cristae and relative size of the mitochondria and the abundance and extent of granal stacking with regard to the chloroplast. In contrast, the mitochondria in the cells in the light-green sectors of cybrid \( 100C \) were strikingly abnormal, and the chloroplasts were also less developed. Mitochondria in these sectors had a greatly reduced inner membrane surface area. In virtually all fields, it was impossible to observe cristae-like structures (Fig. 3C).

The structure of the chloroplast in the light-green sectors was variable. In general the thylakoid area appeared reduced relative to either the wild-type tomato or the dark-green sectors, and in particular there appeared to be fewer and less appressed granal stacks in chloroplasts in the light-green sectors. However, the Chl content was similar in wild-type tomato and the dark-green and light-green sectors of \( 100C \) mutants, 0.57 ± 0.1, 0.89 ± 0.17, and 0.75 ± 0.12 mg/g fresh weight, respectively. Similarly, the Chl \( a/b \) ratios in these same samples were indistinguishable among wild-type tomato and dark-green and light-green sectors of NCV mutants, 1.81 ± 0.13, 2.02 ± 0.2, and 1.99 ± 0.16, respectively. The light-green color of the sectors is apparently the result of the absence of the palisade layer rather than a decreased Chl concentration.

**Characterization of the Chloroplast Genome in Mutants**

The chloroplast genotype of the regenerants had previously been determined using a species-specific HindIII polymorphism (Bonnema et al., 1991). All of the cybrids displayed the tomato-specific RFLP. Usually one or the other parental chloroplast genome is inherited by the fusion products, and no recombination occurs (Galun and Aviv, 1983). To test whether NCV plants were chimeric for ctDNA, total DNA was isolated from dark- and light-green sectors of the variegated leaves of NCV mutant 121, digested with HindIII, and probed. The hybridization patterns of these two sectors were identical; both had the tomato-specific hybridization pattern. To test whether NCV plants had rearrangements at sites other than the HindIII RFLP, ctDNA was purified from the NCV mutant \( 100C \) and from UC82. There were no differences between the restriction patterns of the ctDNA isolated from the mutant and wild type when digested with three different endonucleases (data not shown).
Characterization of the Mitochondrial Genome in Mutants

Commonly, plants regenerated following protoplast fusion have mitochondrial genomes that are a product of recombination between the two parental mitochondrial genomes (Galun and Aviv, 1983). This was also observed in the mitochondrial genome of these tomato cybrids. A set of seven nonoverlapping cosmid clones, representing at least 60% of the tomato mitochondrial genome, was used to probe genomic Southern blots (Bonnema et al., 1991). All NCV mutants had much more mtDNA (60–90%) from *L. pennellii* than the cybrids with normal appearance (6–7% *L. pennellii* mtDNA). Although the cosmids were known to carry specific mitochondrial genes, the nature of the polymorphic DNA sequences (coding versus noncoding) detected by the probes was not known.

To describe the organization and structure of specific mitochondrial genes in NCV mutants, heterologous probes for mitochondrial genes were used. The organization of the mitochondrial genome was investigated around 10 genes: *atpA, atp6, atp8, coxI, coxII, cob, ndh-1, rrn5, rrn18*, and *rrn26* (Table III). Examples of the Southern images scored to produce this table are shown in Figure 4. Initially, species-specific RFLPs were identified between the two parental lines, UC82 and *L. pennellii*, and then the pattern in mutant and nonmutant cybrids was determined. RFLPs were readily detected for all but two of the mitochondrial genes. Purified ctDNA from tomato was included on the Southern blots to demonstrate the specificity of the hybridization signal from the mtDNA in the total DNA preparations.

mtDNA from *L. pennellii* had two hybridizing fragments for *coxII* and *atp8* when digested with *HindIII* (Fig. 4). We independently determined that there were two copies of each of these genes, one on each hybridizing restriction fragment (data not shown). NCV mutants had either parental pattern, usually the *L. pennellii* pattern. In the case of *atp8*, only NCV cybrids inherited the *L. pennellii* forms; the nonmutant cybrids (Fig. 4, lanes 92a and 92b) inherited the tomato forms of *atp8*. In contrast, the tomato form of *coxII* was observed in both an NCV cybrid (Fig. 4, lane 121) and nonmutant cybrids (Fig. 4, lanes 92a and 92b). For 6 of the 10 genes characterized, *atp8, coxI, coxII, nad-1, rrn18*, and *rrn26*, the cybrids had either one or the other parental specific pattern; no novel, nonparental restriction fragments were observed. Frequently, NCV mutants displayed the *L. pennellii* organization for these genes, whereas nonmutant cybrids usually displayed the tomato organization for these genes (Table III).

We were unable to distinguish the parental forms of *atpA* and *cob*. No restriction endonuclease site differences were detected between tomato and *L. pennellii* mtDNA around *atpA* and *cob*. In the case of *atpA*, 15 different endonucleases were tested; in the case of *cob*, 11 endonucleases were screened. Nevertheless, no novel, nonparental fragments were detected in any of the cybrids when *atpA* (Fig. 4) or *cob* was used as a probe.

The structure of the mitochondrial genome around *atp6* and *rrn26* in NCV mutants appeared to be rearranged but still retained a functional function. Restriction digestion of

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Figure 3. Electron micrographs of chloroplasts and mitochondria in cells in the palisade layer of tomato and NCV leaflets. A, UC82. B, Dark-green sector from NCV mutant 100C. C, Light-green sector from NCV mutant 100C. The bar represents 1.0 μm.
the cybrid DNA with enzymes whose sites had been mapped on cloned forms of \textit{atp6} from \textit{L. pennellii} demonstrated that an intact form of the gene was present in NCV mutants (Reiter, 1994). Parental and novel fragments were detected in Southern analyses with \textit{atp6} and \textit{rrn26} only with restriction enzymes producing relatively large hybridizing fragments. Restriction digestions producing smaller fragments demonstrated only parental hybridization patterns. These results are consistent with intergenomic recombinations or rearrangements nearby but not within the genes for \textit{atp6} and \textit{rrn26}.

The tomato mitochondrial genome has been estimated by us and others (McClean and Hanson, 1986) to be approximately 400 kbp or larger. Visual inspection of ethidium bromide-stained restriction enzyme digests of mtDNA purified from tomato, \textit{L. pennellii}, and the cybrids revealed a complex pattern, as expected. There were species-specific differences between the parental lines, and the mutants had fragments from each parental pattern as well as novel, nonparental restriction fragments (data not shown). The ethidium bromide-stained mtDNA images reinforced the summary of the Southern hybridization data (Fig. 4; Table III; Bonnema et al., 1991) but were too complex to indicate specific fragments unique to the NCV mutants.

### DISCUSSION

We have created and characterized cytoplasmically inherited leaf developmental mutants of tomato. Recombination of the cytoplasms from cultivated tomato and \textit{L. pennellii} resulted in plants with abnormal leaf blade development. This phenotype developed independently at least four different times following the fusion of tomato protoplasts with irradiated protoplasts of \textit{L. pennellii}: lines 81, 100A-E, 121, and 122. Either there are a number of molecular events that can cause this phenotype or a single event is favored for some reason, i.e. there is a recombinational hotspot. The NCV phenotype was observed only in tomato plants with cytoplasms generated following protoplast fusion to construct cybrids. Maternal inheritance of this phenotype was formally demonstrated in the progeny of reciprocally crossed tomato and cybrid plants (Table II). Therefore, the location of the gene(s) responsible for the phenotype was proposed to be in either the mitochondrial genome or the chloroplast genome. A similar mutant has been described in tobacco cybrids carrying petunia cytoplasms (Bonnett et al., 1993). The tobacco mutant also lacks a palisade cell layer in the light-green sectors.

The Chl content was not significantly different between the light- and dark-green sectors of NCV mutants or between NCV mutants and wild-type tomato. The ultrastructure of the chloroplasts in the light-green sectors had a variable morphology, which tended to have fewer grana than chloroplasts in the dark-green sectors or in wild-type leaves. Why were the Chl levels not lower in the light-green sectors? The affected sectors of the leaves were found only in the upper half, and all of the cells had chloroplasts. Essentially what we needed to measure was the difference in Chl levels between spongy mesophyll cells and palisade cells in the upper half of spongy mesophyll cells.
We were unable technically to detect any differences. The readily visible leaf color difference was probably due to the cellular and subcellular organization of the palisade layer rather than the abundance of Chl in the sector.

The genome responsible for the NCV phenotype is not likely to be the chloroplast genome. There was no evidence of alterations in the chloroplast genome in mutant plants when restriction enzymes were used to detect rearrangements. The pattern of restriction sites in NCV plants and the parental plant, UC82, was identical when purified ctDNA was examined with three different restriction endonucleases, and there was no difference in the restriction digestion pattern in ctDNA from the light- and dark-green sectors of an NCV plant. NCV plants appear to have normal tomato ctDNA. We cannot rule out the possibility of point mutations in the ctDNA of NCV plants; however, based on the frequency of the NCV phenotype in the population of cybrids, we do not consider this a likely mechanism. We conclude that the abnormal leaf blade development in NCV mutants is the result of one or more mitochondrial mutations or an incompatibility between the recombinant mitochondrial genome and the tomato nuclear or the tomato chloroplast genomes in these plants.

We were unable to identify a molecular basis for the NCV phenotype. For some other mitochondrial mutations like CMS and NCS, molecular mechanisms have been proposed. In the case of several types of CMS, recombinant genes are uniquely present and expressed in the sterile plants (Young and Hanson, 1987; Levings, 1994). In the case of NCS, specific mitochondrial genes are absent in the affected sectors of the heteroplasmic plants (Hunt and Newton, 1991; Roussell et al., 1991; Gu et al., 1993). In addition, there are descriptions of stunting phenotypes similar in part to NCV that have been attributed to nuclear-organellar incompatibility and not necessarily an abnormal or missing mitochondrial gene (Newton and Courtney, 1991; Inai et al., 1993). Whether NCV is the result of an incompatibility between the tomato nuclear genome and the recombinant mitochondrial genome or the result of a specific lesion in a mitochondrial gene or genes is not known.

Newton and Coe (1986) have proposed that the striped variegation pattern in the NCS mutants of corn is the result of heteroplasmic cells in the embryo that segregate during development. Cells with less than a critical number of mutant mitochondria develop normally; cells and their descendants with more than that number of mutant mitochondria develop abnormally, resulting in the stripes. The sectoring pattern of the light- and dark-green regions in NCV mutants also suggests a heteroplasmic state. Leaf blade expansion in dicots results from a mosaic of meristematic sectors (Steeves and Sussex, 1989); therefore, rather than stripes, irregular patches of variegated cells are expected. Our observation that the phenotype becomes more severe as the plants age suggests that the mutant mitochondria are competitive. However, since all of the seedlings with NCV cytoplasm are variegated, and less deformed as young plants, the egg cells must be heteroplasmic. Possibly only heteroplasmic embryos are viable; zygotes homo-plasmic for the mutant mitochondrial genome may be nonviable.

Unlike the NCS mutants in corn, tomato NCV mutants are male fertile. We have reported reduced vegetative and reproductive yields of NCV mutants in field trials (Table I). We predict that the reduction in yield in these plants was a result of reduced photosynthetic activity in the abnormally developed leaves. There might have been a slight effect on fruit yield due to the moderate reduction in pollen viability in the mutants, but the major effect was probably related to the reduced vegetative growth of the plants.

At this point we can suggest three possible molecular bases for the NCV phenotype. First, NCV may be the result of a rearrangement or absence of one or more of the genes we have not tested: coxIII, ndh-2, ndh-3, ndh-4, ndh-5, or ndh-6, one of many tRNA genes, one of several ribosomal protein genes, or any of several unidentified reading frames (Schuster and Brennicke, 1994). Alternatively, the NCV phenotype was not caused by an intergenic recom-

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