Corky Stunt: A Genetic Disease of Tomato

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ABSTRACT

Diagnostic symptoms of corky stunt, a seed-transmitted disease of the tomato cultivar Chico III, and the influcnce of temperature and light intensity on symptom expression are described. Symptoms include shortened internodes, proliferated axillary buds, malformed petioles, roughened lower petiole surfaces, malformed fruit, and corked fruit. Symptoms were most severe, and frequency of symptom expression was highest, at 22.5 C and 43-49 klx with a 16-hour photoperiod. No pathogenic microorganisms were observed in diseased plants, and the disorder was not transmitted mechanically or by grafting. The disease was shown to be controlled by a single recessive gene designated *cst*. A seedling assay technique was developed that used environmentally controlled growth chambers at 22.5 C and 43-49 klx with a 16-hour photoperiod.

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The tomato cultivar Chico III (*Lycopersicon esculentum* Mill.) is grown extensively in the U.S.A. for mechanical harvest. In 1973, a disease of unknown etiology was observed on Chico III plants in several areas of the U.S.A. Symptoms included a severe stunting of plants and a corking and malformation of fruit (W. B. Johnson, Vegetable Crops Specialist, Rutgers University, *personal communication*). In one field in southwestern Indiana, 24% of the plants were affected. The problem recurred in Indiana during 1974 in a field of direct-seeded Chico III where approximately 1-2% of the planting showed symptoms of the disease.

Preliminary studies with seeds collected from affected plants indicated that the disorder was seed-transmitted. Symptom severity was variable on greenhouse-grown seedlings, and appeared to be related to light intensity and temperature. Although symptoms observed in the field suggested the involvement of a pathological agent, the high frequency of seed transmission raised the possibility of a genetically controlled abnormality. This study characterizes the disease as a genetic abnormality controlled by a single gene, and describes the influence of environment on symptom expression.

MATERIALS AND METHODS.—*Sources of seed.*—Tomato seeds obtained in southwestern Indiana from plants showing severe corky stunt symptoms were used as the source of stunted (S) Chico III. Seeds designated normal (N) Chico III were obtained from the Purdue University tomato breeding seed stocks in which the abnormality had not been observed during 5 consecutive years.

Environmental studies.—Seeds from (S) and (N) sources were planted in a soil:sand (3:1,v/v) mixture and watered daily with tap water. Plantings were grown in a greenhouse at 20-25 C for 7 days and moved to growth chambers for observation of the effect of temperature and light intensity on symptom expression. Two temperatures (22.5 C and 30 C continuous) in combination with two light intensities (6-9 klx and 43-49 klx) under a 16-hour photoperiod were used. Seedlings were observed for symptoms for at least 3 weeks after emergence.

Transmission studies.—Various plant species discussed in the Results section were mechanically inoculated by rubbing Carborundum-dusted leaves with Chico III (S) seedling extracts. Extracts were prepared with seedlings triturated in five volumes of chilled buffer [0.5% sodium sulfite or 0.1 M potassium phosphate buffer pH 7.0 containing 0.2% sodium diethyldithiocarbamate (DIECA), 0.1% sodium thioglycolate, and 0.4% 5-mercaptoethanol]. Seedlings were placed in a greenhouse at about 25 C, and observed until maturity for symptom development.

Fig. 1. Corky stunt of tomato cultivar Chico III. Stunting symptom on a susceptible field-grown Chico III plant (A) compared with a symptomless field-grown Chico III plant (B).

We attempted to transmit the disease to symptomless Chico III (N) seedlings by two grafting techniques. Seventeen plants were graft-inoculated by a tissue implantation method. One- to 2-mm sections of stem

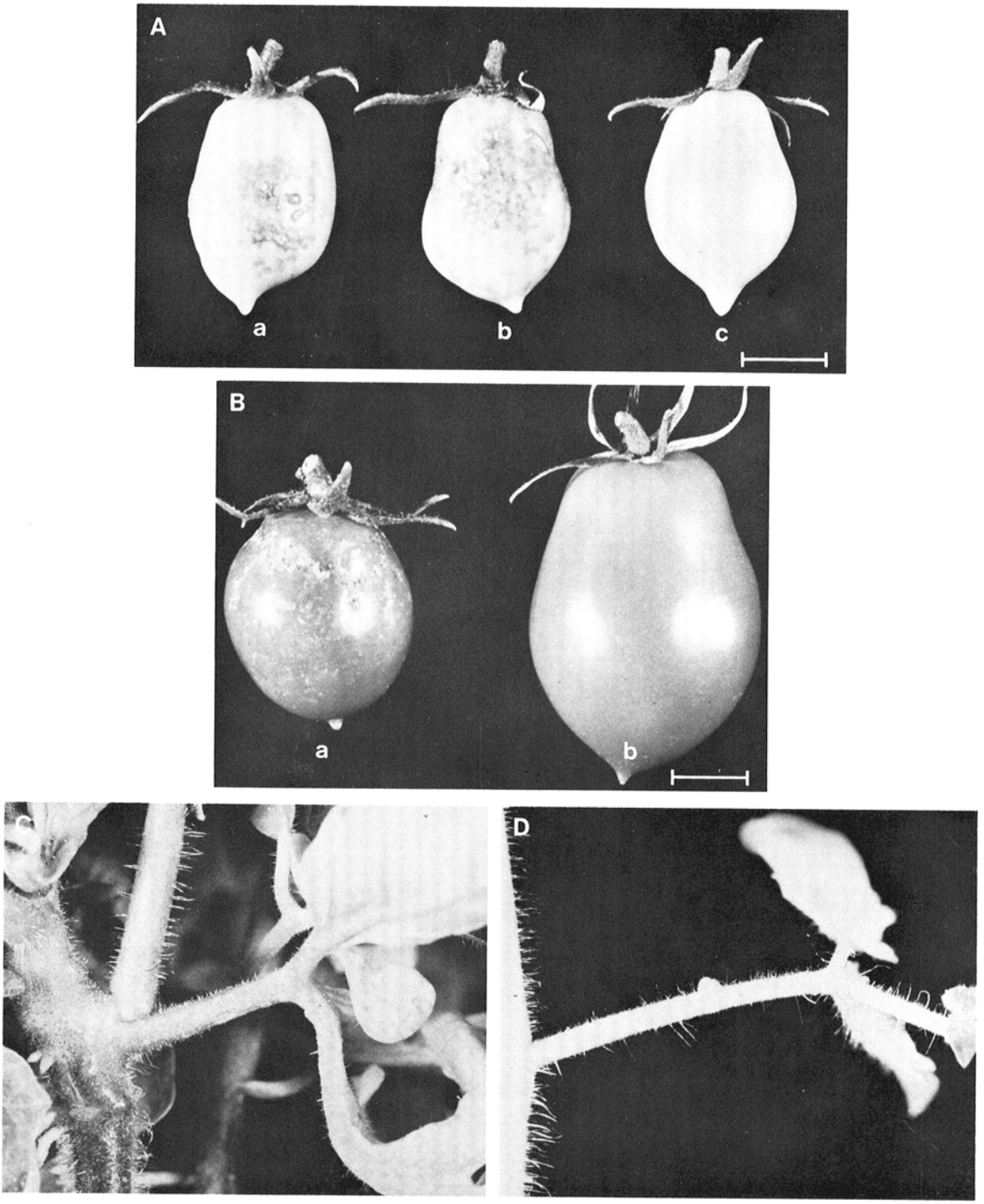

Fig. 2-(A to D). Symptoms of the corky stunt disease on tomato cultivar Chico III. **A)** Immature fruit with incipient fruit lesions (Aa and Ab) compared with a healthy immature fruit (Ac) (scale bar = 1 cm); **B)** Ripe fruit with corky lesions (Ba) compared with healthy ripe fruit (Bb) (scale bar = 1 cm); **C)** Downward angular bending of a lower leaf petiole and enationlike roughening of the lower petiole surface of a plant grown in a high light intensity (43-49 klx), 22.5 C, 16-hour photoperiod environment; **D)** Slight petiole malformation of a plant grown in a low light (6-9 klx), 22.5 C, 16-hour photoperiod environment.

tissue from stunted Chico III (S) seedlings were inserted into the stems of Chico III (N) seedlings. Other grafting tests were conducted by cleft grafting Chico III (N) scions to Chico III (S) root stocks (12 grafts were successful). Foliage from root stocks was periodically removed. Plants were maintained until maturity, either at 22.5 C and 49 klx with a 16-hour photoperiod, or at greenhouse conditions.

Electron microscopy.—Seedlings were grown in a growth chamber at 22.5 C and 49 klx light intensity with a 16-hour photoperiod. Severely malformed petioles with enationlike roughening of the lower surface were selected from Chico III (S) seedlings. Chico III (N) seedlings free of macroscopic symptoms were used as a control. Petiole sections from 3- and 7-week-old seedlings were fixed in 2.5% glutaraldehyde in 0.1 M potassium phosphate (pH 6.8) for 4 hours at 0-4 C. Tissues were postfixed with 1% aqueous OsO_4 in phosphate buffer, stained with 1% aqueous uranyl acetate, dehydrated in acetone and embedded in Luft's Epon. Thin sections were stained with uranyl acetate and lead citrate, and examined in a Philips EM 200 electron microscope.

Genetic studies.—Reciprocal crosses were made with greenhouse-grown cultivars Chico III (S and N) and Lafayette, a symptomless cultivar. Seed from F_1 plants were produced in the field during the summer of 1974. Parents, F_1, and F_2 populations were subsequently grown under conditions required for maximum symptom expression (22.5 C and 43-49 klx with a 16-hour photoperiod) for 28 days and evaluated for symptoms.

RESULTS.—*Symptomatology.*—Symptoms of the disorder on cultivar Chico III were observed in Indiana fields during 1973 and 1974. Diagnostic symptoms included shortened internodes, proliferated axillary buds, a thickened lower stem, malformed lower petioles that were bent downward, and an enationlike roughening of the lower petiole surface (Fig. 1). Corky lesions blemished the fruit surface on affected plants. We propose the term 'corky stunt of tomato' to designate this disorder.

Chico III (S) plants grown in the greenhouse during September to January were free of vegetative symptoms of the disorder. However, up to 69% of these plants showed fruit corking symptoms. Neither fruit nor vegetative symptoms developed on plants grown from the (N) seed source.

Incipient fruit symptoms were dark-green, oval-to-elongated pustules on the immature fruit surface (Fig. 2-A). As fruits matured, the lesions dried, darkened, cracked, and appeared corky (Fig. 2-B). Fruits on a single plant often varied in severity of lesion development. Symptoms on fruit from a single affected plant ranged from an absence of corky lesions to greater than 50% of the surface covered with lesions. Severely affected fruits were abnormally small and distorted. Throughout these studies, fruit corking was less influenced by environment than vegetative symptoms.

Influence of environment on development of vegetative symptoms.—The development of vegetative symptoms was influenced by temperature and light intensity (Table 1). In growth chambers, symptoms were most severe, and typical of field symptoms, in the high light intensity (43-49 klx), 22.5 C environment. Vegetative symptoms under these conditions included downward curvature of petioles of lower leaves (Fig. 2-C), enationlike roughening of the lower surface of malformed petioles, thickened lower stems, and stunted bushy plants which resulted from shortened internodes and proliferated axillary shoots. Roots of diseased Chico III (S) and healthy Chico III (N) plants grown under these conditions were similar in appearance. Milder vegetative symptoms occurred on plants grown under low light intensity at 22.5 C, and under high light intensity at 30 C. The mild symptoms included slight petiole malformation (Fig. 2-D) and slight development of enationlike roughenings on the underside of petioles. Plants grown in these environments were not stunted. No growth abnormalities were observed at low light intensity (6-9 klx) and high temperature (30 C).

Transmission studies.—Chico III (N) plants inoculated by grafting did not develop symptoms of corky stunt, even when grown to maturity. Symptoms were not observed on the following mechanically inoculated plants: *Chenopodium quinoa* Willd.; *Cucumis sativus* L. 'Chicago Pickling' and 'National Pickling'; *Cucurbita maxima* Dcne.; *Curcurbita pepo* var. *melopepo* (L.) Alef.; *Lycopersicon esculentum* Mill. 'Bonny Best', 'Centennial', 'Chico III' (N), and 'Pearson'; *Nicotiana glutinosa* L.; *Nicotiana rustica* L.; Nicotiana hybrid (*N. clevelandii* × *N. glutinosa*) (1); *Phaseolus vulgaris* L. 'Bountiful' and 'Pinto'; and *Vigna sinensis* (Torner) Savi. Chico III (N) seedlings mechanically inoculated with extracts from symptomless grafted, or mechanically

TABLE 1. Effect of light and temperature on symptom expression of the corky stunt disease transmitted through the seeds of tomato cultivar Chico III

Environment[a]		(N) Purdue seed		Seed collected from diseased plants (S)	
Light intensity (klx)	Air temp (C)	Seedlings observed (no.)	Frequency of symptom expression[b] (%)	Seedlings observed (no.)	Frequency of symptom expression[b] (%)
6-9	22.5	27	0	66	28
	30.0	45	0	68	0
43-49	22.5	27	0	69	71
	30.0	137	0	147	20

[a]In environmentally controlled growth chambers with a 16-hr photoperiod.
[b]Average of three trials.

TABLE 2. Inheritance of corky stunt disease

Cross[a]		Progeny phenotype[b] Normal (no.)	Stunted (no.)	P^c (3:1 ratio)
Chico III (N)	⊗[d]	31	0	
Chico III (S)	⊗	1	8	
Lafayette	⊗	38	0	
Chico III (N) × Chico III (S)	F_1	4	0	
	F_2	25	5	.30
Chico III (S) × Chico III (N)	F_1	7	0	
	F_2	22	6	.85
Lafayette × Chico III (S)	F_1	4	0	
	F_2	28	5	.21

[a]Seed sources: cultivars Chico III (N) (=Normal) and Lafayette - Purdue University tomato breeding seed stocks; cultivar Chico III (S((=Stunted) - collected from diseased plants in southern Indiana.
[b]Progeny phenotype evaluated in growth chambers at 22.5 C and 43-49 klx with a 16-hour photoperiod.
[c]Probability of a larger χ^2 assuming a 3 (normal):1 (stunted) ratio.
[d]⊗= Self-pollinated plant.

inoculated, plants did not develop symptoms.

Electron microscopy.—Cells from healthy and abnormal petioles examined by electron microscopy appeared similar, and did not show any obvious cytopathic symptoms. No virus inclusion bodies, mycoplasmalike organisms, bacteria, or other cytopathic abnormalities suggestive of a pathological agent were observed in the cytoplasm of petiolar tissue from (S) or (N) seedlings. Transmission studies and electron microscopic observations suggest that a transmissible agent is not involved in disease etiology.

Inheritance studies.—Inheritance of the disorder in progeny of F_2 crosses of 'Chico III' (N) × 'Chico III' (S) and 'Lafayette' × Chico III (S) fit a 3:1 ratio (Table 2). Transmission through pollen and seed parents minimizes the likelihood of cytoplasmic inheritance. These results support the hypothesis that corky stunt is a genetic abnormality controlled by a single recessive gene. The symbol *cst* (corky stunt) has been approved by C. D. Clayberg of the Coordinating Committee of the Tomato Genetics Cooperative to designate the gene controlling this disorder.

DISCUSSION.—Our results demonstrate that corky stunt is a genetic disease, and that it is not caused by an infectious agent. Inability to detect pathological agents or cytopathic abnormalities by electron microscopy, or to transmit a pathological agent with graft or mechanical inoculation methods, support the results of inheritance studies.

Light intensity and temperature are critical factors in the development of diagnostic symptoms. Lack of control over these factors is presumably the reason for the varied symptoms observed in preliminary greenhouse studies. We propose an environmentally controlled seedling assay in which seedlings containing the homozygous recessive gene, *cst*, can be rapidly identified. Seed samples (minimum of 100 seeds per sample) should be planted in a soil:sand mixture (3:1,v/v) and planting containers maintained in growth chambers set at 22.5 C and 43-49 klx with a 16-hour photoperiod for 21 days. Plants are evaluated at the end of this period for diagnostic symptoms (stunting, petiole malformation, roughening of the lower petiole surface, and axillary bud proliferation). Samples from commercial seedlots of Chico III destined for planting in Indiana in 1975 are currently being tested by this assay at the request of growers. Of the limited number of samples tested thus far, corky stunt has been detected in a single seedlot (10% of seedlings affected). We have been unable to detect corky stunt in breeders' seed obtained from the originator of the Chico III cultivar.

There is precedent for genetically controlled abnormalities in commercial tomato seedstocks. The gold fleck and fruit pox syndromes are controlled by the dominant gene *(Gdf)* and recessive gene *(fp)*, respectively (2). These abnormalities, which differ in symptom expression from corky stunt, have been observed in tomato cultivars in several areas of the United States (2). Development of gold fleck and fruit pox symptoms, however, did not appear to be affected by seasonal or environmental conditions, and thus differ from corky stunt.

The high frequency of corky stunt, which is a simply inherited genetic abnormality, in certain 1973 commercial seedstocks raises the question of when this potentially serious disorder arose, and how it escaped detection until first reports in 1973. Since corky stunt was absent in the breeders' seeds that were tested, it is probable that a mutation arose early in commercial seed increase, and that the environmental conditions necessary for optimum symptom development were absent. A genetic abnormality expressed only under specific environmental conditions poses a unique problem in seed certification. Present commercial seedstocks and breeders' material should be tested in an environmentally controlled seedling assay to prevent further yield losses and eliminate the corky stunt gene from breeding stocks.

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