Resistance of Tomato Ripening Mutants and Their Hybrids to *Botrytis cinerea*

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ABSTRACT

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Fruits and peel extracts of nonripening *nor* and *rin* tomatoes suppressed germ-tube elongation in *Botrytis cinerea*, as compared with mature normal fruits. Similar levels of suppression also were seen with mature-green normal fruits. Contact inoculation with a diseased tomato fruit was used to determine the relative resistance of the mutant fruits and their hybrids to *Botrytis* infection. Resistance was indicated by the prolongation of the incubation period necessary for infection and the reduced incidence of rot during storage. The highest level of resistance was shown by the *nor* mutant and its F_1 hybrid, suggesting that this character was transferred from the mutant to the hybrid fruit. Exposure to 0 C for 3 days or to hot water (52 C) for 5 min before inoculation markedly increased the susceptibility of normal fruits and partially broke the resistance of the previously resistant fruits. We suggest that the relative resistance of the nonripening mutants and the *nor* hybrid to infection by *B. cinerea* is diminished by environmental conditions that favor penetration. The results also suggest the presence in the mutant tomato fruit of factors that suppress conidia germination and may be involved in their resistance to infection.

Storage life of normally ripening cultivars of tomato (*Lycopersicon esculentum* Mill.) is limited by both fruit softening and the development of postharvest diseases. However, fruits of the nonripening *rin* and *nor* tomato mutants fail to soften and have a long shelf life (9–11). Lacking the normal pigmentation of tomato fruit, these mutants are of limited value in their homozygous state, but crossing the mutant with a normal ripening cultivar results in F_1 hybrids that produce fruits characterized by both color development and a much reduced rate of softening, as compared with fruits from normal cultivars (4,7). In addition, both mutants and their hybrids are reported to be much less susceptible to postharvest rots than normal fruits (2,3,5,9).

Our work examined the resistance of mutant fruits and their hybrids to infection by *Botrytis cinerea* Pers.:Fr., one of the main pathogens of tomato fruit (1,6), by studying differences in conidia germination, infection, and lesion development among normal, mutant, and hybrid fruits. Because contact with a decayed fruit serves as a common mechanism for disease initiation in harvested tomato fruit (6), we tried to simulate conditions of natural infection.

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MATERIALS AND METHODS

The following genotypes were grown in the open field in Israel: normal tomato cultivar (n-5-F8-321-2), its partially isogenic nonripening *nor* and *rin* mutants (n-5-F8-307-b and n-5-F8-297-b, respectively, which are F8 plants with the *nor* and *rin* loci in a heterozygous condition), and F_1 *nor* and *rin* hybrids (*nor*/+ and *rin*/+)—the result of a cross between the two. The fruits were picked at two stages of maturity: mature-green and mature. The mature stage for the nonripening fruits and their hybrids was defined as about 14 days after their "turning point," characterized by the changing of peel color from green to yellow or to red.

Conidia germination in peel extracts and fruit tissues. For studies of conidia germination in fruit peel extracts, 50-g aliquots of fresh peel tissue (about 1 mm thick) of the normal and the mutant fruits at two stages of development were removed and homogenized for 1 min in 150 ml of 95% ethanol with a Sorval Omnimixer. The filtered residue was washed twice with ethanol, and the filtrate was concentrated twice in vacuo at 40 C, then partitioned twice with dichloromethane, as described by Prusky et al (8). The organic phase, designated as peel extract, was dried with anhydrous $MgSO_4$ and concentrated in vacuo.

Aliquots of 50 µl of the organic extracts were applied on 13-mm-diameter Millipore disk filters (0.45-µm pore size) on glass slides in three replicates. After ethanol evaporation, each disk was loaded with 10 µl of an aqueous suspension (10^5 conidia per milliliter) also containing 5% ethanol, 0.05% Tween 20, and 0.0015% dimethyl sulfoxide, as described previously (8).

The conidia suspension was prepared from a 7-day-old monoconidial culture of *B. cinerea* on PDA. The origin of the culture was a decayed tomato fruit.

After 4 hr of incubation in a moist chamber at 18 C, the conidia were fixed in lactophenol-cotton blue. Millipore disks with 50 µl of ethanol but without peel extracts and loaded with aliquots of the conidia suspension after ethanol evaporation served as controls.

Conidia germination within the normal and the *nor* mutant fruits also was examined. Millipore disks loaded with 50 µl of a 10^5/ml conidia suspension were inserted into a 4-mm^2 cut in the pericarp of mature-green and mature fruits beneath the skin. The cut was then recovered with the fruit skin and sealed with cellophane tape. Fruits were incubated for 4 hr at 18 C, and the conidia were scraped from the tissue and fixed as above.

Each experiment was done in three replicates. Percent germination and germ-tube elongation were examined microscopically in nine replicate samples of 100 conidia each.

Inoculation and lesion development in normal, mutant, and hybrid fruits. Normal, mutant, and hybrid fruits were inoculated at two stages of maturity: 1) by inserting into the pericarp, through a 4-mm-deep wound, a uniform-sized inoculum consisting of agar disks with both mycelium and conidia cut with a 4-mm cork borer from the periphery of a 7-day-old monoconidial culture grown on PDA at 18 C and 2) by placing healthy fruits in contact with a diseased mature normal tomato covered with mycelium and conidia. Some fruits inoculated by the contact procedure were exposed to chilling and heat treatments by being held for 3 days at 0 C or for 5 min in hot (52 C) water before inoculation.

Five replicate fruits were used for the insertion inoculation and 30 for the contact inoculation, and each experiment was repeated twice. The inoculated fruits were held at 18 C and 90–95% RH in controlled storage rooms. Radial rate of rot development after inoculation with mycelium and conidia and incidence of infection after inoculation by contact

with a rotting fruit were determined daily for 7 days.

RESULTS

Conidia germination in peel extracts and fruit tissues. Percent germination in peel extracts of the mature *rin* and *nor* fruits did not differ significantly from that recorded in peel extracts of the normal fruit or in the control disks that lacked peel extract. However, rates of germ-tube elongation were significantly lower in mature *nor* and mature-green normal fruit than in mature *rin* and mature normal fruit. Mean germ-tube lengths in the mature normal fruit samples were similar to those in the no-extract control samples (Table 1).

Incubation of conidia within ripe normal tomato resulted in significantly higher rates of germ-tube elongation than incubation within mature-green normal fruit or *nor* mutant fruit at the two stages of maturity. No significant differences in percent germination were found among fruit types (Table 2).

Infection and lesion development in normal, mutant, and hybrid fruits. After inoculation into the pericarp, rate of development of *B. cinerea* was slower in the *nor* mutant fruit than in the *rin* fruit and the normal fruit during the initial stages of host colonization. This was expressed by prolongation of the incubation period of the disease and by subsequent decreased lesion diameter during the holding period. For each fruit, lesion diameter increased with maturity (Fig. 1).

After contact inoculation with a diseased tomato fruit, the *nor* mutant and its hybrid were both markedly more resistant to infection than the normal and the *rin* fruits. This effect was more pronounced at the mature-green stage, requiring 5 and 3 days of incubation, respectively, for symptoms to appear (*data not shown*). In addition to the prolongation of the incubation period necessary for infection, the relative resistance of the *nor* mutant and its hybrid was also expressed by a significantly reduced incidence of decay after 7 days (Table 3).

Exposing fruits to chilling temperature or to hot water treatment before inoculation generally increased the incidence of decay during storage (Table 3). Although these pretreatments increased the susceptibility of the *nor* mutant and its hybrid to decay, their resistance, as compared with that of normal fruit and of the *rin* mutant and hybrid, was still retained. Further, whereas the rot had spread over normal fruit by the end of the holding period, lesions were more restricted on the *nor* mutant and its hybrid. A similar restricted decay was noted in the *rin* mutant and its hybrid, although incidence of infection in these fruits was similar to that in the normal fruit (*data not shown*).

DISCUSSION

Our results indicate important levels of resistance to *B. cinerea* in the mutant tomato fruits and mainly in the *nor* fruits, although they do not clearly indicate the nature of that resistance.

Extracts of the peel of mutant fruits suppressed elongation of *B. cinerea* germ tubes but not conidia germination. The inhibitory effects on germ-tube elongation were confirmed in vivo, when conidia were placed within fruit tissues of the *nor* mutant at both the mature-green and the mature stage. The rate of germ-tube elongation for *nor* mutant mature-green and mature fruits as well as for normal mature-green fruits was about half that for normal ripe fruits. Lack of marked differences between percent germination of conidia in different fruits at different stages of maturity is in agreement with the similar rates of germination recorded by Verhoeff and Liem (13) for conidia of *B. cinerea* incubated in the sap of small (2- to 3-cm-diameter) green or ripe fruits of a normal tomato cultivar.

The prolongation of the incubation period detected in the *nor* mutant after inoculation of *B. cinerea* into the pericarp of the fruit at both stages of maturity is probably the result of the inhibitory effect of the mutant tissues to the initial stages of hyphal growth. After initial colonization, however, the rate of fungal development in the mutant fruit was similar to that in the normal fruit. Contact inoculation with a diseased tomato fruit was a good means for indicating the relative resistance of the *nor* mutant fruit and its hybrid to infection by *B. cinerea*, as compared with all the other genotypes tested. The highest level of resistance occurred in the *nor* mutant. The resistance of the *nor* hybrid, as compared not only with that of the normal fruit but also with that of the *rin* mutant and its hybrid, suggests that this character was transferred, to a large extent, from the *nor* mutant to its F_1 hybrid.

The resistance of the *nor* mutant and

Table 1. Percent conidia germination and germ-tube elongation of *Botrytis cinerea* in peel extracts of normal and mutant tomato fruit[a]

Source of fruit peel extract	Percent germination	Germ-tube length (μm)
Mature-green normal	76.5 ± 3.1[b]	32.6 ± 3.5
Mature normal	80.3 ± 2.8	49.9 ± 4.7
Mature *rin*	78.4 ± 2.1	40.4 ± 4.1
Mature *nor*	74.5 ± 4.4	28.9 ± 2.2
Control[c]	78.2 ± 2.1	48.5 ± 2.2

[a]After 4 hr at 18 C.
[b]Standard error of the mean.
[c]Conidia germination in the absence of extracts.

Table 2. Percent conidia germination and germ-tube elongation of *Botrytis cinerea* in normal and *nor* tomato fruit at two stages of maturity[a]

Fruit type	Percent germination	Germ-tube length (μm)
Mature-green normal	84.9 ± 4.7[b]	18.4 ± 0.5
Mature normal	89.3 ± 3.1	36.1 ± 2.1
Mature-green *nor*	83.8 ± 5.2	18.2 ± 1.0
Mature *nor*	85.7 ± 6.6	19.7 ± 1.1

[a]After 4 hr at 18 C.
[b]Standard error of the mean.

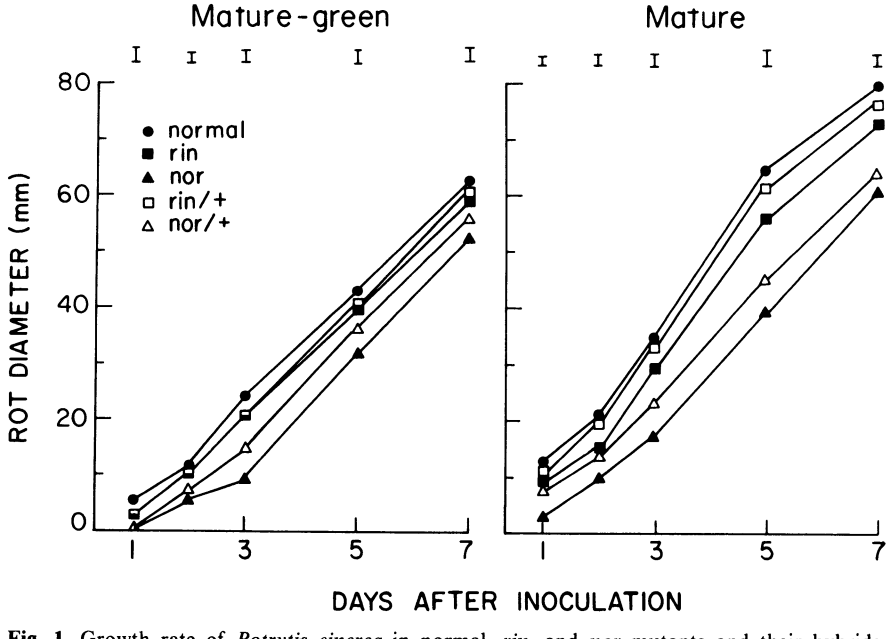

Fig. 1. Growth rate of *Botrytis cinerea* in normal, *rin*, and *nor* mutants and their hybrids after inoculation into the pericarp. Inoculation was done by inserting, through a 4-mm-deep wound, a uniform-sized agar disk of a 7-day-old monoconidial culture on PDA at 18 C. Vertical bars denote standard errors of the mean.

Table 3. Incidence of *Botrytis cinerea* decay in normal tomato fruit and in *rin* and *nor* mutants and their hybrids (*rin*/+ and *nor*/+) at 18 C after contact inoculation with a diseased fruit or after exposure to extreme temperatures before contact inoculation

Stage of maturity / Fruit genotype	Incidence of decay (%)[a] after					
	2 days			7 days		
	Control	Prechilled[b]	Preheated[c]	Control	Prechilled	Preheated
Mature-green						
Normal	5.2 ± 1.1[d]	8.6 ± 1.1	9.9 ± 1.2	54.6 ± 1.3	64.7 ± 1.6	70.9 ± 1.3
rin	4.6 ± 1.3	5.9 ± 2.2	9.8 ± 1.6	49.8 ± 2.1	64.3 ± 1.3	69.3 ± 1.4
nor	0	0	0	5.4 ± 1.3	49.1 ± 1.7	59.4 ± 1.3
rin/+	4.9 ± 1.9	5.6 ± 1.3	8.6 ± 1.4	53.8 ± 1.4	66.2 ± 1.6	69.7 ± 1.2
nor/+	0	0	5.9 ± 1.3	14.9 ± 1.3	49.6 ± 1.4	59.6 ± 1.3
Mature						
Normal	14.8 ± 1.3	14.9 ± 1.6	19.8 ± 1.3	80.0 ± 2.1	90.2 ± 2.4	100
rin	9.8 ± 1.1	9.9 ± 1.6	19.7 ± 1.2	75.0 ± 2.4	84.6 ± 3.7	95.0 ± 2.1
nor	0	0	7.7 ± 1.4	14.7 ± 2.0	65.0 ± 2.2	68.7 ± 2.4
rin/+	9.3 ± 1.9	9.6 ± 2.1	19.8 ± 1.2	73.8 ± 1.2	85.5 ± 1.2	94.8 ± 2.3
nor/+	0	0	9.9 ± 1.6	24.6 ± 1.3	69.8 ± 2.1	74.3 ± 2.1

[a]Each value is the average of 30 replicates.
[b]Exposure to 0 C for 3 days before inoculation.
[c]Exposure to hot (52 C) water for 5 min before inoculation.
[d]Standard error of the mean.

nor hybrid to infection by *B. cinerea* when placed in contact with a diseased fruit suggests an association with the response of the periderm of these fruits to fungal penetration. This suggestion was further supported by the results obtained from exposing the fruits to extreme temperatures before inoculation. Both chilling and high temperature treatment partially broke the resistance of the *nor* fruit and its hybrid to infection by *B. cinerea*, resulting in increased incidence of decay during storage. Chilling temperatures are known to predispose the cold-sensitive tomato fruit to infection by *B. cinerea* (6) and were previously found to increase the susceptibility of the *nor* mutant to infection by *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind (2). Hot water treatment, which removes the natural wax layer from various parts of the tomato surface (12), seems to increase the susceptibility of the fruit to infection by preparing suitable sites for fungal penetration. The ability of the two preinoculation treatments to decrease the differences in susceptibility between the normal and the mutant fruits may support the assumption that the relative resistance of the mutant fruit and its hybrid is associated, to a large extent, with the ability of the pathogen to penetrate the fruit. Some suppression of fungal growth in the resistant fruit seems to be directly related to the effect of peel and pericarp tissues on initial stages of hyphal growth, but once this barrier is breached, invasion of the underlying tissues is rapid.

The resistance of *nor* and *nor* hybrid fruits to invasion by *B. cinerea* is important in the commercial marketing of tomatoes, where nesting is a common problem (6) and where fruits in transit are often exposed to environmental conditions favorable for fungal development.

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