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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (3): 774-777, 1999 Research Article

# Identification of a New Dependable Tomato Differential in Tomato Late - Blight Pathosystem

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## Abstract

The sensitivity of 15 lines of tomato (*Lycopersicon esculentum*) to six isolates of the late blight fungus (*Phytophthor infestans*) was assessed by a detached leaflet method at several plant ages. Most lines showed variation in reaction different ages; FMX-93 and LA 1338 were consistently sensitive to all isolates whereas LA 1623 was hypersensitive to isolates except Ca65 which attacked these and all other lines. Isolate 88/15 showed consistent virulence to most tomat lines and avirulence to LA 1623 and Rockingham. Accessions Rockingham and LA 1623 were crossed to susceptib accessions. When screened with isolate 88/15, all $F_1$ plants of both crosses were resistant. $F_2$ families of each cro segregated to produce approximately 25 percent susceptible plants. A single dominant gene conferring resistance is thu indicated in each resistant parent. This was confirmed in backcross families which segregated 1:1 for resistanc susceptibility.

## Introduction

The major problems with using virulence/avirulence phenotypes as markers are: lack of a standardized virulence - assessment method; the race of an individual isolate might change during culture or in storage (Ery *et al.*, 1992); and non-existence of a sufficiently well-characterized series of differentials (Michelmov and Hulbert, 1987). The need for genetically well defined partners to probe the mechanistic and molecular basis for host - parasite relationships has often been stressed. The objectives in these experiments were (1) to identify isolates with stable pathogenicity on both potato and tomato for use in later studies (2) to identify stable resistance in tomato (3) to determine the inheritance pattern of stable late-blight resistance identified in tomato accessions.

## Materials and Methods

**The Pathogen**: The isolates used in these studies were collected in UK, Mexico and USA and were stored cryogenically as part of the *P. infestans* culture collection at the University College of North Wales, Bangor. Isolates were maintained over short periods on slants of rye A agar (Caten and Jinks, 1986) in screw-top Mccartney bottles under heat-sterilized light paraffin mineral oil (BDH Chemicals Ltd.) at 18°C in the dark. Sporangia for inoculation were harvested from 10 to 15-day-old petri dish cultures by adding 10 ml sterile pond water.

**The Host**: In the first greenhouse experiment, tomato lines used were: FMX-93 and LA-1338 which were susceptible; West Virginia 63, 700, New Yorker and Rockingham were known to have some resistance. Accessions LA 1511 and LA 1621 with unknown resistance were also used.
In the second greenhouse experiment, tomato lines used were: FMX-93, West Virginia 63 and Nova. In addition, six other lines (LA 1620, LA 1623, LA 1456, LA 41 Roughby) with unknown resistance to the pathogen we used.

**Growing of the test plants**: Four tomato seeds were plant in each 13 cm diameter pot and maintained in t glasshouse at a day temperature of 18 ± 3°C and nig temperature of 12 ± 2°C with natural sunlight augment by 40 W sodium-vapour lighting on a 14 h light, 10 h da cycle. Four plants per genetic line were transplanted sing into 20 cm diameter pots after one month. Plants w grown in a peat-sand compost made with Chempak Potti Base. Pots were placed on a capillary mat which w wetted every day. One month after planting, a Chempa liquid fertilizer (No 4: NPK 15:15:30) was given weekly the plant at the recommended rate of 2 L per week. Mat leaflets were selected from fully expanded leaves fr plants of different ages for testing against *P. infestans*.

**Inoculation of leaflets**: The method as described by Kherb (1988) was used.

Inheritance study: Resistant accessions LA 16 Rockingham and susceptible accessions, LA 1420 FMX-93 were used in this experiment. Four tomato se of each accession were planted in soil-less compost in a cm diameter pot in the glasshouse (day temperature 25 ± 2° C and night temperature of 20 ± 2°C) with nat sunlight augmented by 40 W sodium vapour lighting 16 h-day. Four plants from each accession w transplanted singly into 20 cm diameter pots after month. Pots were placed on a capillary mat which wetted every day. One month after planting, a Chem fertilizer (No 4) NPK 15:15:30 was given (two L per weekly to the plants at the recommended dilution. Cro were made in the greenhouse from May through June.

potato isolate 88/15, and classified as tomato race O was used in all tests for segregation. The two susceptible lines (FMX-93, LA 1420) and one susceptible line (FMX-93) were crossed with resistant lines Rockingham and LA 1623 respectively. Eight-week old $F_1$ seedlings (nine plants for each cross) were tested for resistance to tomato race O. Nine $F_1$ plants were allowed to self-pollinate to produce $F_2$ seeds and four $F_1$ plants for each cross were test-crossed with the two susceptible lines as shown in Table 3. F2 and test-cross progenies were raised in 10 cm diameter pots, one plant per pot. Test-cross and $F_2$ population sizes of at least 100 plants were screened with isolate 88/15. Two mature leaflets from each seven to eight-week-old plant were inoculated as described earlier. Leaflets from parents of the same age were used as controls. Chi-square tests for goodness-of-fit were used (the correction factor of 0.5 was subtracted from the absolute value of the difference between observed and expected) for analysing the data from the segregating populations (Fisher and Yates, 1963).

## Results and Discussion

The Host: Both experiments gave a clear indication that FMX-93 allowed rapid development of disease when inoculated with all six isolates (Tables 1, 2). If this line carried any specific resistance, it was not detected by the isolates used. Non-specific resistance in this line was low. Accession LA 1338 reacted in a similar way to FMX-93.

WV 700 was attacked in experiment 1 by all isolates but gave an incompatible reaction at one of the two ages with three isolates (Table 1). Gunther *et al.* (1970) reported that WV 700 carried a gene for high field resistance and proposed the gene-symbol Phf for its field resistance. The present results suggest that there was no stable specific resistance of a hypersensitive type in this accession. If gene(s) for specific resistance existed in WV 700 then either they were not expressed in the material tested or all isolates had complementary virulence(s) to the gene(s). Turkensteen (1973) designated the partial resistance of WV 700 as $Ph_2$ based on single-gene inheritance. It has been proposed that nomenclature Ph should be used only for specific resistance and not for partial, non-specific resistance (Gallegly, 1960).

In experiment one New Yorker, LA 1511, LA 1621, WV-63 and Rockingham showed a clearer differential response to the isolates than the other genotypes (Table 1).

Table 1: The interactions between eight lines of tomato and six isolates of pathogen.

| Cultivar or line | Isolates of pathogen | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 550 | | 88/15 | | 88/1 | | 87/33 | | $89AF_1$ | | Ca65 | |
| | 17 week | 19 week | 17 week | 19 week | 17 week | 19 week | 17 week | 19 week | 17 week | 19 week | 17 week | 19 week |
| FMX-93 | + | + | + | + | + | + | + | + | + | + | + | + |
| LA1338 | + | (-) | + | + | + | + | + | + | + | + | + | + |
| WV700 | + | - | + | + | - | + | + | - | + | + | + | + |
| New Yorker | - | - | - | - | - | + | - | + | + | + | + | + |
| LA 1511 | - | - | - | - | - | + | - | - | + | + | + | + |
| LA 1621 | - | (-) | - | - | - | + | - | + | (-) | + | + | + |
| WV 63 | - | (-) | - | - | - | - | - | - | + | - | + | + |
| Rockingham | + | - | - | - | - | - | - | - | - | - | + | + |

+ compatible; - incompatible; (-) intermediate (sparse sporulation)

Table 2: The interactions between nine lines of tomato and six isolates of pathogen.

| Cultivar or line | Isolates of pathogen | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 550 | | | | 88/15 | | | | 88/1 | | | |
| | 7 week | 9 week | 11 week | 13 week | 7 week | 9 week | 11 week | 13 week | 7 week | 9 week | 11 week | 13 week |
| FMX-93 | + | (-) | + | + | + | + | + | + | + | (-) | + | |
| LA 411 | - | + | (-) | - | + | + | + | - | - | | - | - |
| LA 1620 | - | - | + | + | - | - | - | - | - | - | - | |
| NOVA | - | - | - | - | - | - | | - | - | - | - | - |
| LA 1623 | - | (-) | - | - | - | - | - | - | - | - | - | - |
| LA 1456 | - | - | (-) | (-) | - | - | - | - | - | (-) | - | - |
| LA 1546 | - | - | - | - | - | | - | - | - | - | - | - |
| Roughby | - | - | + | - | - | - | - | - | - | - | - | - |
| WV 63 | - | (-) | + | - | - | | - | - | - | (-) | - | - |

| | 87/33 | | | | 89AF1 | | | | CA65 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 week | 9 week | 11 week | 13 week | 7 week | 9 week | 11 week | 13 week | 7 week | 9 week | 11 week | 13 we |
| FMX-93 | + | + | + | + | + | + | + | + | + | + | + | + |
| LA 411 | + | + | - | (-) | + | (-) | - | + | + | + | + | + |
| LA 1620 | + | (-) | - | - | + | - | - | - | + | + | + | + |
| NOVA | - | - | (-) | - | - | - | - | - | + | + | + | + |
| LA 1623 | - | - | - | - | - | - | - | - | + | + | + | + |
| LA 1456 | - | - | - | - | - | - | - | - | + | + | + | + |
| LA 1546 | - | - | (-) | - | + | + | - | - | + | + | + | + |
| Roughby | - | (-) | - | - | - | - | - | - | + | + | + | + |
| WV 63 | - | - | (-) | - | (-) | - | (-) | - | + | + | + | + |

+ compatible; - incompatible; (-) intermediate (sparse sporulation)

Table 3: Late-blight resistance and susceptibility of $F_2$ and testcross populations of tomato crosses between lines resist and susceptible to 88/15.

| Population | Number of individuals | | Expected | | |
|---|---|---|---|---|---|
| | Resistant | Susceptible | ratio* | $X^2$ | P |
| $F_2$ Population FMX-93 (F) x LA 1623 (M) | 70 | 30 | 3:1 | 1.08 | 0.50-0 |
| Rockingham (F) x LA 1420 (M) | 74 | 26 | 3:1 | 0.01 | 0.95-0 |
| Rockingham (F) x FMX-93 (M) | 71 | 29 | 3:1 | 0.65 | 0.50-0 |
| Testcross Population | | | | | |
| (FMX-93 x LA 1420) x LA 1420 (F) (M) | 52 | 48 | 1:1 | 0.09 | 0.95-0 |
| (Rockingham x LA 1420)x LA 1420 (F) (M) | 43 | 57 | 1:1 | 1.69 | 0.50-0 |
| (Rockingham x FMX-93) x FMX-93 (F) (M) | 51 | 49 | 1:1 | 0.01 | 0.95-0 |

* expected on the basis that resistance is determined by a single gene. (F) = female; (M) = male.

In either experiment, lines carrying resistance gene (Ph-1), i.e. New Yorker, WV-63, Rockingham and Nova (Gunther *et al.*, 1970; Spielman *et al.*, 1989) did not give the same spectrum of interaction or similar reactions with the age of the plant or gave slight sporulation on detached leaflets (Tables 1,2). It was suspected that the different reactions with age and light sporulation on detached leaflets (Nova, WV-63) may be because the lines carried both specific and non-specific resistance to late-blight (Gallegly and Marvel, 1955). In both experiments, WV-63 showed resistance to all isolates except Ca 65 although interactions with some isolates indicated weak expression of resistance. Only host Rockingham gave consistent reactions on five and eight-week-old plants. Further increase in age tended to increase susceptibility; this is in line with the observations already made by Walter and Conover (1952) for non-specific resistance. Analysis of accession-isolate interactions has led to the identification of a truly dependable accession LA 1623. This is reported here, for the first time. The assessment of dependability was based on the fact that resistance was consistently expressed at all ages tested. Al-Kherb's (1988) results were supportive to the extent t he tested 48 tomato accessions including LA 1623 aga 10 isolates including Ca 65 and that LA 1623 was the accession that gave incompatible reactions to nine isol and compatible reaction to only one isolate (Ca 65). Fur experiments on inheritance of resistance are conducte tomato hosts FMX-93, Rockingham and LA 1623 ident in these investigations.

**The Pathogen:** Physiological races of a pathogen can be distinguished on the response of a corresponding s differential hosts. If resistance in Rockingham and LA 1 is due to the single gene Ph-1 then these lines disting two virulence phenotypes (two races), one vir (virulence phenotype 1 or race 1) and one avir (virulence phenotype 0 or race 0). Only one isolate, C isolated from tomato in California, attacked all host and is designated race 1 assuming that resistant lines only one resistance gene, Ph-1 (Tables 1, 2). The isolates tested were avirulent on resistant hosts designated race 0.

These isolates were made from potato from several countries. Isolate 88/15 gave leaf, reproducible reactions on a number of tomato hosts (Table 1 and 2). Thus this isolate was used as representative of tomato race 0 in inheritance study.

**Inheritance of resistance:** Crosses of LA 1623 and Rockingham, both showing a hypersensitive reaction to isolate 88/15, to susceptible FMX-93 and LA 1420 gave rise to $F_1$ populations which were all resistant. $F_2$ and backcross populations segregated susceptible phenotypes in a proportion not significantly different from 25 and 50 per cent, respectively (Table 3).

These data suggest that resistance in both LA 1623 and Rockingham is determined by a single, dominant gene, that parents were homozygous and $F_1$ individuals were heterozygous. The result is consistent with the gene-for-gene hypothesis and suggests that isolate 88/15 carries an avirulence gene which is complementary to the resistance gene of each host.

A gene, Ph-1, conferring hypersensitivity to some isolates has been identified in several cultivars, e.g. New Yorker, Nova, West Virginia Accessions 36, 106, 700, 63 (Walter, 1967; Gunther *et al*., 1970).

These results showed that the currently available Ph-1 tomato differential did not given reproducible results. These studies resulted in finding the LA 1623 as the new most dependable tomato differential with Ph-1 gene.

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