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Short communication

Vegetative compatibility among isolates of *Colletotrichum gloeosporioides* from almond in Israel

Talma Katan and Ezra Shabi
Department of Plant Pathology, ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel (Fax: 972-3-9683543; 972-3-9604180)

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Abstract

Anthracnose, caused by *Colletotrichum gloeosporioides*, is the major disease of almond in Israel. Pathogen attack of young fruit results in fruit rot and leaf wilting. Seventy isolates of *C. gloeosporioides* were obtained from affected almond fruits collected at 11 sites during 1991–2 and 1994. Chlorate-resistant nitrate-nonutilizing (*nit*) mutants were generated from each isolate and used in complementation (heterokaryon) tests. The formation of complementary stable heterokaryons between mutants from different isolates showed that all the isolates belonged to a single vegetative compatibility group. Representative isolates of *C. gloeosporioides* from almond did not form heterokaryons with local isolates of *Colletotrichum* from anemone and avocado, indicating that the almond isolates constitute a distinct subspecific group within *C. gloeosporioides*.

In Israel, almond (*Prunus amygdalus* Batsch.) is grown mainly in two regions: the Yizre'el Valley and Lower Galilee in the north, and the Judean foothills and coastal plain in the south. Anthracnose, caused by *Colletotrichum gloeosporioides* Penz., is the major disease of almond in Israel. The disease was first recorded in 1977 in a few orchards in the northern and southern regions and reached an epidemic level in subsequent years. Pathogen attack of young fruits results in fruit rot and desiccation as well as leaf wilting of distal clusters and, in extreme cases, shoot dieback, even though the pathogen was not detected in leaves or twigs (Shabi and Katan, 1983). Disease control relies primarily on preventive fungicide sprays, although studies on cultivar resistance have been initiated (Striem et al., 1989).

Species of *Colletotrichum* are important pathogens of several crops in Israel; *C. gloeosporioides* is the major pathogen of avocado, causing post-harvest fruit rot (Binyamini and Schiffmann-Nadel, 1972), and *Colletotrichum* sp. is the major pathogen of anemone, causing the leaf-curl disease (Katan and Shabi, 1994). The relatedness of these pathogens to *C. gloeosporioides* from almond is not known. *C. gloeosporioides* is a complex species, encompassing diverse groups of strains and biotypes (Sutton, 1992). Taxonomic classification of *Colletotrichum* spp. has been based primarily on host preference and morphological characteristics. The value of these criteria is limited by the variation and stability of the traits, which are sensitive to environmental conditions, and the existence of intermediate forms. Studies of vegetative compatibility offer another approach to determining genetic relatedness in anamorphic populations of *Colletotrichum* (Chacko et al., 1990; Correll et al., 1993a, b; Wasilwa et al., 1993; Katan and Shabi, 1994). Since hyphal anastomosis is a pre-requisite for the exchange of genetic material, isolates belonging to the same vegetative compatibility group (VCG) are expected to be genetically more similar to each other, thus constituting a distinct subspecific group within the 'species' complex (Leslie, 1993). The objective of this study was to determine genetic relatedness among the populations of *C. gloeosporioides* from almond, and between the almond pathotype and other pathotypes of *Colletotrichum*. The study was based on previous experience with pathogenic *formae speciales*

Table 1. Isolation of *Colletotrichum gloeosporioides* from almond fruits

Region	Site code[1]	Number of isolates		
		1991–2	1994	Total
North	GOZ	3	14	17
	GVA	1		1
	GZT	2		2
	IKS		9	9
	KN	3		3
	KSH	1		1
	KYZ	3		3
	YAF	2		2
South	BZR	2	3	5
	NA		3	3
	NRB	1	23	24
Total	11 sites	18	52	70

[1] GOZ: Givat Oz; GVA: Geva; GZT: Gazit; IKS: Iksal; KN: Kafr Kanna; KSH: Kfar Kish; KYZ: Kfar Yehezkel; YAF: Yafia; BZR: Bizzaron; NA: Nahshon; NRB: Nir Banim.

of *Fusarium oxysporum* (Katan et al., 1991, 1994), using nitrate-nonutilizing (*nit*) mutants to test vegetative compatibility, as suggested by Puhalla (1985).

Seventy isolates of *C. gloeosporioides* from almond, originating from 11 sites at two geographic regions, were used in this study. The 18 isolates from the first sampling period (1991–1992) originated from seven northern and two southern sites, each site being represented by 1–3 isolates. The 52 isolates from the second sampling period (1994) originated from three of the previous sites and two additional sites, one in the north and one in the south (Table 1). *C. gloeosporioides* was isolated from anthracnose-affected almond fruits as described previously (Shabi and Katan, 1983) and one monoconidial culture was then prepared from each isolate. Cultures were maintained on potato dextrose agar (PDA). Most of the isolates had a light-grey aerial mycelium and their growth rate at the optimal temperature (20–22 °C) was about 2.2 mm day^{-1}. A few isolates had a flat white mycelium and grew slightly faster. Monoconidial isolates of *Colletotrichum* from anemone and avocado (Shabi et al., 1993; Katan and Shabi, 1994) were included for comparison.

The *nit* mutants were generated as previously described by Puhalla (1985). Petri dishes (9-cm diameter) containing minimal nitrate agar (MM), amended with asparagine and 15 g l^{-1} potassium chlorate ($KClO_3$), were centrally inoculated with small mycelial plugs and incubated at 20–22 °C. Fast-growing sectors emerging from the restricted colonies were transferred to Petri dishes (5 cm diameter) containing MM and examined after a 4-day incubation period. Colonies with a thin expanding mycelium were considered *nit* mutants. The first sectors were evident about 10 days after inoculation. The sectors varied in shape and colour (Figure 1); their number ranged from one to four averaging two per colony. The *nit* mutants were isolated from about two weeks after inoculation to about two months thereafter and were partially phenotyped by growing them on media containing nitrate, nitrite, hypoxanthine or ammonium as the nitrogen source (Correll et al., 1987; Brooker et al., 1991). Mutants at the nitrate reductase structural locus (*nit1*) comprised 52% of the total number of mutants, mutants at a nitrate-assimilation pathway-specific regulatory locus (*nit3*) comprised 24%, and mutants at loci involved in the biosynthesis of a molybdenum-containing cofactor (NitM) comprised 24%. In addition, some sectors yielded chlorate-resistant colonies that grew profusely on MM; many of these mutants were unable to grow on nitrite medium and were considered leaky *nit3* mutants. Similar chlorate-resistant nitrate-utilizing mutants (designated *crn1*) of *Fusarium moniliforme*, found closely linked to the *nit3* locus, were presumed to be leaky alleles at this locus (Klittich and Leslie, 1989). Other such mutants might be deficient in nitrate and chlorate uptake (Unkles, 1989), or have originated from genetically impure sectors. None of the nitrate-utilizing mutants of *C. gloeosporioides* was used in complementation tests.

Complementation between *nit* mutants was tested on MM. Generally, each plate was inoculated with three mutants in a triangle formation, and incubated at 20–22 °C. Complementation was usually evident within 10–15 days by the formation of a dense aerial wild-type mycelium where mycelia from two mutants met and formed a prototrophic heterokaryon (Brooker et al., 1991; Katan et al., 1991). Absence of wild-type growth at the contact zone between two *nit* mutants of the same parent isolate indicated allelic, overlapping, or otherwise non-complementary mutations, or vegetative self-incompatibility (Brooker et al., 1991). On the other hand, absence of wild-type growth at the contact zone of *nit* mutants from different parent isolates indicated either non-complementarity or lack of vegetative compatibility. When mutants of two different isolates formed a heterokaryon, their parents were assigned to the same VCG. The mutants of each of the 18 isolates of the first group (7–21 mutants per isolate) were first paired among themselves to deter-

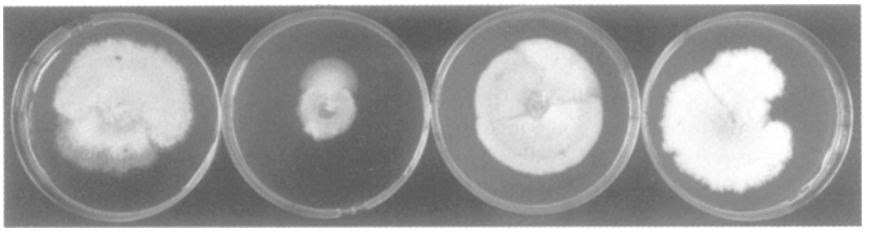

Figure 1. Formation of chlorate-resistant sectors by isolates of *Colletotrichum gloeosporioides* growing on nitrate minimal medium amended with $KClO_3$: top view of isolates (left to right) ALM-IKS4F, -IKS5L, -IKS7Q, and -NA3P. One month after inoculation.

mine complementation within isolates. Representative, preferably complementary mutants of each isolate were then paired in all possible inter-isolate combinations to determine complementation and vegetative compatibility. In these pairings, all mutants formed stable complementary heterokaryons with at least some of the other mutants. Complementation was strongest in pairings involving a NitM mutant. Complementation also occurred in some pairings between *nit1* and *nit3* mutants or among some *nit1* mutants, but not among *nit3* mutants. The pattern of complementary heterokaryon formation indicated that all 18 isolates belonged to a single VCG. Mutants of the 52 isolates of the second group (1–8 mutants per isolate) were paired in various intra- and inter-isolate combinations and with representative NitM mutants of the first group. Formation of complementary heterokaryons indicated that all isolates of the second group belonged to the VCG defined by the first group. No vegetative self-incompatibility was found among the 70 isolates examined. Complementation among 46 NitM mutants from 36 isolates was determined in about 300 pairwise combinations. Four complementation groups were found, comprised of 14, 13, 13 and six NitM mutants; though this number of complementation groups is small, it still falls within the range of 4–7 groups found in several fungal species (Tomsett and Garrett, 1980; Klittich and Leslie, 1988; Newton and Caten, 1988; Unkles, 1989; Debets et al., 1990). Any pair of NitM mutants from different complementation groups could serve as dependable testers for the VCG of *C. gloeosporioides* from almond.

Complementary mutants from almond isolates were paired with *nit* testers representing the local population of *Colletotrichum* from anemone (Katan and Shabi, 1994) and with complementary *nit* mutants of three *C. gloeosporioides* isolates from avocado (T. Katan, unpublished). No complementation was observed in these pairings. The almond isolates differ from the anemone and avocado isolates in growth rate, optimal growth temperature and sensitivity to agricultural fungicides (Shabi et al., 1993). Recent studies, applying molecular criteria, have provided further evidence that the almond pathotype constitutes a genetically uniform, subspecific clone of *C. gloeosporioides*, distinct from the avocado pathotype (Freeman et al., 1996).

The origin of the almond pathotype in Israel is not known; initial occurrence appears to have happened several years prior to the first record in 1977, allowing for spread and establishment in both almond-growing regions. Anemone and avocado can evidently be excluded as possible sources. *Colletotrichum* spp. are occasionally isolated from diseased ornamental plants, but their appearance is sporadic and confined to nurseries maintaining a humid environment. In the past, bitter rot caused by *Colletotrichum* sp. (*Gloeosporium fructigenum* Berk.) was a destructive disease of apples in Israel (Pappo, 1965) but, following the replacement of overhead sprinkler irrigation with drip irrigation, it has not been recorded since the late 1970s. Growth rate and optimal temperature reported for the apple bitter-rot isolates were higher than those of the almond isolates. In some Mediterranean regions of southern Europe, anthracnose diseases have been recorded on almonds and olives (cited by Shabi and Katan, 1983, and Graniti et al., 1993, respectively), but no comparative studies have been performed between the respective *C. gloeosporioides* pathotypes. Anthracnose has not been observed on olives in Israel. Isolates of *C. gloeosporioides* from olive resemble those from almond in their ability to cause disease on immature green fruits, but differ by their faster growth rate, slightly higher optimal growth temperature and ability to infect vegetative plant organs (Graniti et al., 1993). Vegetative compatibility should prove useful in determining relatedness among the *C. gloeosporioides* pathotypes of almond, apple and olive.

Classification of *Colletotrichum* strains by morphological and physiological criteria may lead to uncertain

conclusions due to environmental influence on the stability of such traits (e.g. Daigle and Cotty, 1994). Categorization by host of origin or host preference may be inaccurate because a given host may be colonized by more than one *Colletotrichum* species (Freeman and Rodriguez, 1995) or biotypes (Liyanage et al., 1992). Vegetative compatibility allows for distinguishing anamorphic strains of *Colletotrichum* and classifying them by genetic means independent of morphology, physiology or pathogenicity. This approach should be useful in identifying and delineating subspecific genetic clones within *C. Gloeosporioides* as well as other complex species of *Colletotrichum*.

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