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Full Length Research Paper

Differentiation of olive *Colletotrichum gloeosporioides* populations on the basis of vegetative compatibility and pathogenicity

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Twenty five isolates of *Colletotrichum gloeosporioides* obtained from fruits of olive, apple and citrus trees from different regions of Golestan province, northern Iran. Results from cross-inoculation experiments showed a great variability in pathogenicity among the isolates examined. They were investigated using complementation tests with nitrate-non-utilizing (Nit) mutants to know their vegetative compatibility. Among 250 chlorate-resistant sectors obtained, only 187 were Nit mutants. Three types of Nit mutants were obtained (*Nit1, Nit3* and *NitM*) on the basis of the fungal phenotype. *Nit1* mutants were the most frequent (71.6%), followed by *NitM* (16.6%) and *Nit3* (11.8%). Based on their ability to form heterokaryons, all olive pathogenic isolates were grouped into two vegetative compatibility groups (VCG). This is a good indication of the homogeneity of the olive *C. gloeosporioides* population. The results might also suggest the absence of a relationship between pathogenicity of strains on apple and VCG.

Key words: Nit mutants, VCG, *Colletotrichum gloeosporioides*, Olive tree.

INTRODUCTION

Olive (*Olea europaea* L.) is the most important and traditional woody crop that is cultivated over a large areas in Iran. Olive cultivation has expanded during the last decade especially in Golestan province, the northern Iran (Figure 1). In this province nearly 10,000 hectare of olive orchards are present, which represents about 20% of total national olive area (Anonymous, 2007). In the last decade, most of the new plantations in this region, established with Rooghany, Zard and Mary cultivars, are the native olive cultivars of Iran (Sanei et al., 2004). Commercial cultivars of olive are planted in Iran but wild olive are the important genetical sources of olive, residue of them can be seen in the East of Golestan province, north of Iran (Sanei et al., 2005).

Unfortunately, olive is subjected to be attacked with a variety of fungal pathogens, which affect its health, yield and its oil quality (Sanei et al., 2011). Anthracnose caused by *Colletotrichum acutatum* J.H. Simmonds and *Colletotrichum gloeosporioides* (Penzig) Penzig and Saccardo is a common, widespread disease of olives in most olive-growing regions in the world, causing pre-and post-harvest problems (Sergeeva et al., 2008). The disease was first recorded in Iran by Sanei et al. (2005) from northwest of Iran in Roodbar and Golestan province, northen Iran (Gilan province). Olive anthracnose, also known as 'lebbra', 'gaffe' or other trivial names, which is responsible for considerable losses of olive crops is the most serious disease of olive in the humid areas (Graniti and Laviola, 1981). On its first appearance in Italy, the pathogen caused up to 80 to 100% loss of yield in some areas and extensively damaged olive trees (Graniti et al., 1993). The infected fruits showed nearly circular lesions, 5 to 7 mm diameter, depressed and brown in colour. Occasionally there was some wilting of younger shoots on the edge of the tree crown. Infection of flowers leading to fruit rot is of economic importance as anthracnose results in significant losses in yield (Talhinhas et al., 2005) and in the quality of oil produced from fruits infected with *C. gloeosporioides* (Iannota et al., 1999). Incubation of the surface-sterilised flowers showed that they were infected from early stages of flowering until fruit set and that the fungi were present in calyx, petals,

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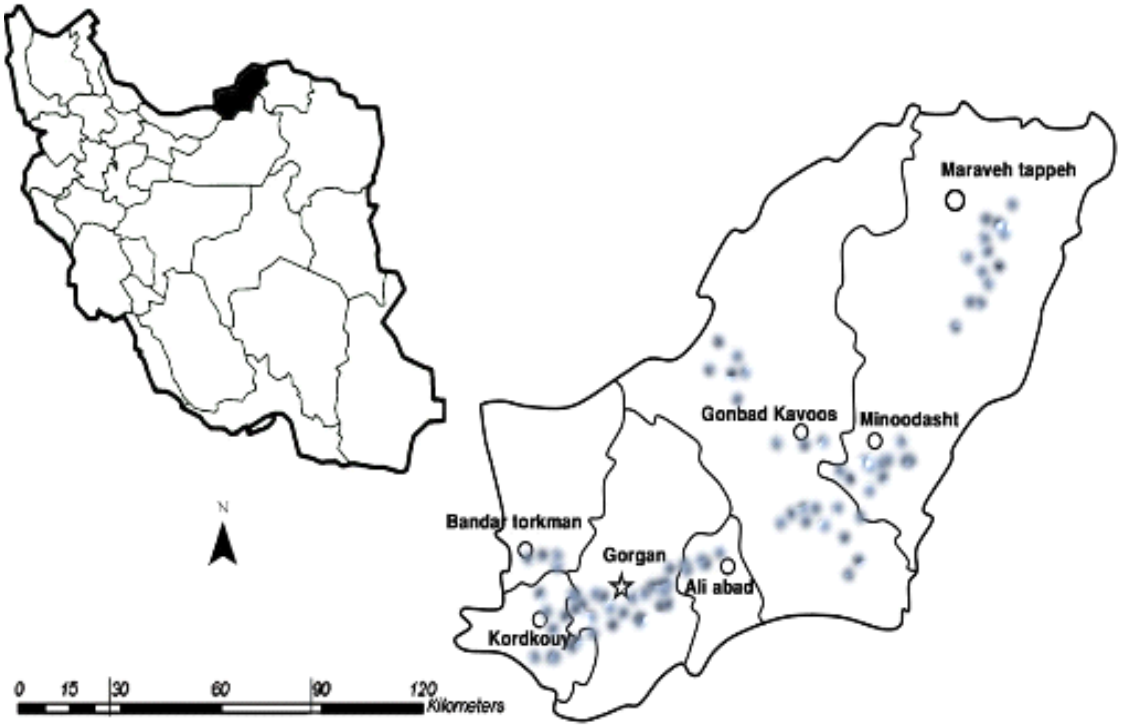

Figure 1. Iran map (left) and regional surveyed (Golestan province, right) in this study.

stamens and pistil. In some instances the pedicels were also infected. The first external symptoms occurred after fruit set on immature fruits when they were at peppercorn (2 to 4 mm) and pea (5 to 10 mm) sizes. Some fruit drop can occur at both these stages and the fruits that remain on the trees can exhibit sporulating colonies of *C. acutatum* and *C. gloeosporioides* (Sergeeva et al., 2008).

C. gloeosporioides, which is considered as a group species, is genetically heterogeneous, with homo- and heterothallic strains, and shows high variability because of its heterokaryotic condition (Van der Aa et al., 1990). Chromosomal rearrangements, occurring during the somatic growth of the fungus or through parasexual processes, may contribute to its variability (Masel et al., 1990). A variety of approaches to species or race separation have been evaluated, including isozyme analysis and electrophoretic patterns of conidial proteins. Ribosomal DNA has been also used as a taxonomic marker for *C. gloeosporioides* (Braithwaite et al., 1990). Strains and populations of *C. gloeosporioides* specialized on various hosts, as well as geographical races, are known. Differences between isolates are also evident with respect to their relative pathogenicity or virulence.

Cross-inoculation studies with *C. gloeosporioides* showed host specificity in a range of isolates tested on a variety of hosts (Jeffries et al., 1990). However, only few studies have been made with cross inoculations of isolates from olive (Ciccarone, 1950).

Vegetative compatibility is a genetically determined ability in which isolates of the same fungal species anastomose and form stable heterokaryons, during which genetic material may transfer from one isolate to another, also the formation of heteroplasmons and subsequent parasexual genetic exchange and transmission of mycoviruses and virus-like agents (Leslie, 1993). Isolates belonging to a VCG tend to have a common genetic pool (Leslie, 1993) and, therefore, may have similar pathological and physiological traits differing from those of isolates that do not belong to that VCG (Joaquim and Rowe, 1991; Nitzan et al., 2006, 2002). Isolates which are vegetatively compatible may form subspecific populations that tend to be genetically isolated in nature and are called vegetative compatibility groups (VCGs). Studies on a range of ascomycetes have shown that vegetative compatibility is generally controlled by a series of genes variously termed vic (vegetative incompatibility) or het (heterokaryon incompatibility) genes. Typically, hyphal fusion occurs between strains irrespective of their genotype, but a killing (incompatible) reaction occurs if the strains differ at one or more of their vic loci. Usually, multiple (up to ten) vic loci are present in a given species, with each locus existing in two alleles. Strains that are able to form heterokaryons are considered to belong to the same vegetative compatibility group (VCG). Studies, especially in the genus *Fusarium,* have shown the usefulness of nitrate non-utilising mutants *(*nit mutants) for demonstrating the existence of VCGs in fungal populations (Correll et al., 1987, 1991). In many fungal

species such mutants can be readily selected as spontaneous sectors on a medium amended with chlorate, a toxic nitrate analogue. The technique has been used in *Colletotrichum* (Brooker et al., 1991; Correll et al., 1993*).* In Iran it has been recorded from almost 50 hosts, and is a significant pathogen of apple, olive, and citrus fruits (Sanei et al., 2011). As part of a molecular study of the nature of pathogenesis in this species we have investigated the extent of vegetative compatibility between a diverse range of these isolates to better understand the population structure.

Testing isolates for VCGs usually is based on complementation tests with nitrate- non-utilizing (nit) mutants (Korolev and Gindin, 1999; Korolev and Katan, 1997), which are impaired in their ability to utilize nitrate from the growing media. When the hyphae of two different nit mutants anastomose to form stable heterokaryons, a biochemical complementation takes place in the heterokaryons which allows them to utilize nitrate when it is the sole nitrogen source in the growing media. The result of the complementation is visible to the naked eye as a prototrophic growth at the contact zone of the mutants' colonies. However, the formation of heterokaryons and, consequentially, the biochemical complementation and prototrophic growth are contingent upon the vegetative compatibility of the isolates from which the mutants were derived. If the isolates were vegetatively incompatible, stable heterokaryons would not have been formed and a prototrophic growth would not have been evident. Hence, testing isolates for vegetative compatibility using nit mutants is a technique based upon a biological response which genetically is restricted, and its results tend to be clear-cut (Leslie, 1993).

Nitzan et al. (2006) collected 110 isolates of *C. gloeosporioides* from European and Israeli potato tubers, roots, and stems and tested their vegetative compatibility. In all, 36 isolates were from Israel, 23 from France, and 51 from the Netherlands. The study resulted in the characterization of four multimember VCGs among the 110 isolates, and the selection of 12 different isolates as VCG testers for the European/ Israeli population (Nitzan et al., 2002). The results indicated that the subspecific level of *C. gloeosporioides* may significantly affect disease epidemiology and etiology and, consequentially, disease management; and strengthened the hypothesis that differences in aggressiveness could be correlated with differences in vegetative compatibility or with the subspecific level of the pathogen. Isolates from different VCGs may possess different epidemiological traits; therefore, a thorough understanding of the diversity in the population of *C. coccodes* would have an important and practical contribution to the improvement of disease management.

When sexual recombination does not occur, isolates within a VCG tend to be more similar than isolates in different VCGs. Isolated VCGs act as genetically isolated lineages that can evolve divergent pathogenicity and vegetative viability traits (Tsror (Lahkim) and Hazanovski, the genetic structure of populations of plant-pathogenic fungi, including *Fusarium oxysporum* (Puhalla and Hummel, 1983), *Verticillium* spp. (Joaquim and Rowe, 1991; Puhalla and Hummel, 1983; Sanei et al., 2008), and *Colletotrichum* spp. (Brooker et al., 1991; Nitzan et al., 2006). In most cases, analysis of VCGs has been based on pairings between complementary nitrate auxotrophic (Nit) mutants of different isolates, selected using chlorate-containing agar. Compatible isolates, whose complementary Nit mutants can form stable heterokaryons, are assigned to the same VCG (Korolev and Katan, 1997). The usefulness of VCGs as a diagnostic tool in plant pathology already has been demonstrated, at least for plant pathogens that do not regularly recombine (Tsror (Lahkim) and Hazanovski, 2001). Due to the disease's increasing economic impact on fruit trees worldwide, studying the structure of populations of *C. gloeosporioides* has become more important. Understanding the diversity within this species may contribute to a better understanding of the epidemiology of the disease and improve its control (Botseas and Rowe, 1994).

As anthracnose is a common, widespread disease of olives in most olive-growing regions in the world, limited data about the pathogen, the diversity and variability of pathogenicity of the *C. gloeosporioides* population (as the major pathogen in north of Iran) were considered. The hypotheses of the current study were that there is VCG diversity within *C. gloeosporioides* subpopulations and that *C. gloeosporioides* isolates from different VCGs possess different virulence to olive. Our specific objectives were to (i) examine and compare VCG diversity and composition among *C. gloeosporioides* isolates collected from olive in north of Iran and (ii) evaluate the aggressiveness of different VCGs to olive, to determine whether there are differences between representatives of these (presumably asexual) VCGs.

MATERIALS AND METHODS

Fungal isolates

Isolation of the fungi was carried out by collecting infected olive, apple and citrus trees (8 to 10-year-old) in Golestan province, the northen Iran (Figure 1). *C. gloeosporioides* were isolated by surface sterilising fruits with 1% sodium hypochlorite for 2 min followed by washing three times in sterile distilled water. The fruits were placed on potato dextrose agar in 80 mm Petri dishes and incubated for 2 days at 25°C. Strains were recovered by transfer of visible conidial masses or tissue from the margin of lesions on rotting fruit to potato-dextrose agar (PDA, Difco) amended with streptomycin (40 mg/L). All were single-spored by spreading conidia onto half-strength PDA made to 3% agar, and isolating individual germlings after 16 h using the procedure of Constantinescu (1988). Monoconidial cultures were maintained on C'zapek dox agar (CDA, Difco) at 6°C (Korolev and Katan, 1997). The origin of the strains is indicated in Table 1. Conidia for microscopic examination and

Table 1. Host and location of selected isolates of *Colletotrichum gloeosporioides*.

Isolates	Host	Location	Conidial size[a]			Growth on PDA (mm/day)[b]	Lesion area (cm^2)[c]
			Length	Width	Length/ width		
C1	Olive	Gorgan	15.3±2.50 a	4.6±0.6 ab	3.4 a	7.0 d	7.7 b
C2	Olive	Gorgan	15.5±2.19 a	4.6±0.4 ab	3.4 a	9.3 b	11.0 a
C3	Olive	Minoodasht	14.3±2.70 a	4.6±0.3 ab	3.1 a	8.5 c	10.6 a
C6	Apple	Gonbad kavoos	15.5±2.70 a	5.6±0.3 a	2.8 b	11.6 a	3.0 c
C8	Olive	Gorgan	15.1±2.23 a	4.6±0.2 ab	3.2 a	9.5 b	11.0 a
C11	Olive	Gorgan	15.6±2.50 a	4.6±0.4 ab	3.3 a	9.6 b	7.5 b
C13	Olive	Gorgan	15.1±2.30 a	4.6±0.3 ab	3.2 a	7.4 d	3.5 c
C15	Olive	Kordkouy	15.1±2.42 a	4.6±0.5 ab	3.2 a	7.4 d	7.4 b
C18	Olive	Kordkouy	14.3±2.6 ab	4.6±0.4 ab	3.2 a	9.4 b	11.0 a
C19	Olive	Kordkouy	14.5±2.3 ab	4.5±0.3 ab	3.2 a	9.6 b	3.0 c
C20	Olive	Kordkouy	14.3±2.7 ab	4.4±0.6 ab	3.2 a	7.0 d	1.5 d
C23	Olive	Gorgan	14.6±2.2 ab	4.4±0.4 ab	3.1 a	7.6 d	1.5 d
C24	Olive	Gorgan	14.6±2.2 ab	4.4±0.6 ab	3.1 a	9.4 b	7.3 b
C26	Citrus	Gorgan	15.0±2.70 a	5.6±0.3 a	2.6 b	11.6 a	7.7 b
C27	Olive	Minoodasht	14.0±2.3 b	4.0±0.2 b	3.2 a	8.4 c	7.4 b
C28	Olive	Minoodasht	14.0±2.5 b	4.4±0.3 ab	3.1 a	8.4 c	1.5 d
C29	Olive	Gonbad kavoos	14.0±2.3 b	4.5±0.4 ab	3.2 a	7.3 d	3.0 c
C30	Olive	Kordkouy	14.0±2.5 b	4.0±0.3 b	3.3 a	7.3 d	1.5 d
C31	Olive	Kordkouy	14.0±2.5 b	4.6±0.2 ab	3.2 a	9.4 b	10.2 a
C33	Olive	Gorgan	13.2±2.6 c	4.1±0.3 b	3.2 a	7.3 d	3.6 c
C34	Olive	Gorgan	13.3±2.5 c	4.1±0.5 b	3.2 a	7.4 d	10.1 a
C35	Apple	Kordkouy	15.0±2.70 a	5.4 ±0.3 a	2.7 b	11.3 a	3.8 c
C36	Apple	Kordkouy	15.4±2.70 a	5.6±0.3 a	2.7 b	11.7 a	3.0 c
C37	Citrus	Minoodasht	15.1±2.70 a	5.4±0.3 a	2.8 b	7.1 d	11.0 a
C38	Olive	Gonbad kavoos	12.3 ±0.8 d	4.0±0.3 b	3.1 a	7.3 d	10.2 a

a Each value is the mean of 100 measurements. Values followed by the same letters are not significantly different by Duncan's multiple-range test (P = 0.01). µm±standard deviation. b Measurements taken from 7-day old colonies grown in Petri dishes in the dark, at optimum temperature (25°C). c Measurements taken 7 days after inoculated apples of cv. Golden Delicious by inserting a mycelial plug into a hole made by a cork-borer and incubated at 25°C. Each value is the mean of six replicates. Values followed by the same letters are not significantly different by Duncan's multiple-range lest (P= 0.01).

measurement were taken from PDA and on inoculated fruits. One hundred conidia were measured for each strain.

Pathogenicity tests

Pathogenicity tests were performed on apples (cv. Golden Delicious) using the needle technique. Apples were washed under a gentle stream of tap water for 10 min, sterilized for 10 min. A sterilized scalpel was used to make cuts 5 mm long and 3 mm deep into the pulp of the fruits and the cut was filled with conidial masses from 20 day-old colonies. The inoculated fruits were placed separately in sealed plastic bags and kept at 25°C for 7 days.

Nit mutant selection

Water agar chlorate (WAC) medium, containing 2% agar, 3% potassium chlorate, and 0.02% glucose) was used to generate Nit mutants. 10 mycelia plugs (1 mm) placed from monoconidial cultures of each isolate on WAC medium and incubated these plates at 27°C in the dark. After 21 days of

incubation, growing edges of samples were transferred onto plates containing CDA medium and allowed them to grow for 5 days. Colonies with a thin mycelium on CDA plates were considered Nit mutants. Partial phenotyping of Nit mutants (*nit1*/*nit3* versus *NitM*) were carried out by placing two mycelial plugs of each isolate on both CDA and CDA amended with 0.02% hypoxanthine (Brooker et al, 1991; Martelli, 1960). Plates were incubated for 5 days at 27°C in the dark. Colonies that grew on CDA supplemented with hypoxanthine with a wild-type phenotype, and on CDA with a thin mycelium, were classified as *nit1*/*nit3* mutants. Colonies that grew with a thin mycelium on both media were classified as *NitM* mutants.

Complementation tests

Complementation between Nit mutants was tested on CDA medium containing sodium nitrate as the nitrogen source. One mycelial block (1 mm) of *NitM* and two mycelial blocks of *nit1* were placed 1 cm apart in a triangular pattern (Martelli, 1960). Plates were incubated for 14 days at 27°C in the dark. Complementation usually was evident after 10 to 14 days, characterized by prototrophic growth at the contact zone between the two complimentary Nit mutants. During all tests, isolates were represented by the same Nit mutant repetition from their collection (either *NitM* or *nit1*).

RESULTS

Some characteristics of several isolates of *C. gloeosporioides* from olive in Golestan province were compared with those of isolates from other hosts (citrus, apple) growing in the same area. The sizes of conidia produced in culture by all isolates were within the range reported for *C. gloeosporioides* (Mordue, 1971). Conidia from olive, however, formed a quite homogeneous group which could be morphologically distinguished from those from other hosts on the basis of a higher length/breadth ratio and shape (Table 1). When the growth rates of the various isolates were compared at 25°C, some isolates of *C. gloeosporioides* grew much more slowly (Table 1). Results from cross-inoculation experiments showed a great variability in pathogenicity among the isolates examined. For example, isolates from non-olive were less virulent on apples than isolates from olive hosts (except for one from citrus) (C37 in Table 1).

All isolates of *C. gloeosporioides* produced numerous chlorate-resistant sectors, but only 1 to 12 Nit mutants were obtained from each isolate. Among the 250 chlorate-resistant only 187 (74%) were characterized as Nit. 134 (71.6%) were characterized as *Nit1*, 31 (16.6%) as *NitM* and 22 (11.8) as *Nit3.* These averages showed a strong disparity from one strain to the other. The investigation of Nit mutants using the isolates (C3, C15, C31 and C38) gave only *Nit1* mutants. The *NitM* was obtained by 21 strains from the 25 used (Table 2). All possibilities of complementations between the *Nit*M of the 21 strains and *Nit*1 or *Nit*3 of the all strains were done. The results showed that all the *C. gloeosporioides* strains coming from the four different hosts were assembled in three vegetative compatibility group (Table 3). The isolates originating from non-olive were distributed among VCGs1 and olive isolates were assigned in two VCGs (VCG2 and VCG3). There was a relationship between pathogenicity of olive and non-olive isolates and VCGs. Colonization levels of *C. gloeosporioides* in inoculated apple fruit were significantly highest for VCG1 isolates and lowest for VCG2 for olive isolates. Colonization levels were different for non-olive isolates (Table 1).

DISCUSSION

Species of the genus *Colletotrichum* cause anthracnose on a wide range of crops, in tropical and subtropical countries but also elsewhere (Sergeeva et al., 2008). The most common species on olive fruits is *C. gloeosporioides* (Sanei et al., 2005) a ubiquitous and polyphagous species shows high variability in morphological characters and in pathogenicity (Martelli, 1959; Mordue, 1971). Fruit rot caused by the pathogen is the most serious disease of olive in the humid areas like Calabria (southern Italy) characterized by environmental conditions favouring epidemic development (Graniti et al., 1993; Martelli, 1959). The *C. gloeosporioides* complex is well known to exhibit high variability in conidial size and shape and in colony morphology (Masel et al., 1990). Conidia from olive, however, formed a quite homogeneous group which could be morphologically distinguished those from other hosts on the basis of a higher length/breadth ratio and shape. The variability here recorded among three isolates of *C. gloeosporioides* from olive underlines once more what was pointed out in a review by Van der Aa et al. (1990).

Inoculations were performed on green apples, which are a common host of *C. gloeosporioides.* All isolates produced well developed (0.5 to 1 cm) necrotic areas in 6 days. After 10 days, *C. gloeosporioides* isolates from olive and especially from orange had produced rotting of the tissue around the inoculation site (diameter: 3 to 4 cm for the olive isolates, 5 cm for the orange isolate) which was covered with blackish grey mycelium, with a few acervuli producing the conidial masses. The results were similar for olive *C. gloeosporioides* isolates with other workers (Mugnai et al., 1993; Sergeeva et al., 2008). The pathogenicity tests continue to be important in taxonomic studies in the *Colletotrichum* complex because of the high specificity of the fungus as shown by the response of different fruits inoculated with homologous isolates (Mugnai et al., 1993).

VCG analysis based on generating Nit mutants on WAC medium (Korolev and Katan, 1997) resulted in recovery of at least one Nit mutant for each isolate (Table 2). Therefore, WAC medium was found suitable for generation of Nit mutants in *C. gloeosporioides*. In the present study, we identified two phenotypic classes of Nit

Table 2. Number of each *Nit* mutant type selected on characterization media.

Isolates	*Nit*1[a]	*Nit*M	*Nit*3	Total
C1	5 (62.5)	1 (12.5)	2 (25)	08
C2	8 (72.7)	2 (18.2)	1 (9.1)	11
C3	1 (100)	0 (0)	0 (0)	01
C6	5 (62.5)	1 (12.5)	2 (25)	08
C8	8 (88.8)	1 (11.2)	0 (0)	09
C11	2 (40)	1 (20)	2 (40)	05
C13	7 (58.3)	3 (25)	2 (16.7)	12
C15	7 (100)	0 (0)	0 (0)	07
C18	4 (80)	1 (20)	0 (0)	05
C19	8 (72.7)	1 (9.1)	2 (18.2)	11
C20	9 (81.8)	2 (18.2)	0 (0)	11
C23	8 (66.7)	3 (25)	1 (8.3)	12
C24	7 (70)	1 (10)	2 (20)	10
C26	8 (88.8)	1 (11.2)	0 (0)	09
C27	3 (75)	1 (25)	0 (0)	04
C28	5 (71.4)	2 (28.6)	0 (0)	07
C29	3 (75)	1 (25)	0 (0)	04
C30	4 (80)	1 (20)	0 (0)	05
C31	6 (100)	0 (0)	0 (0)	06
C33	6 (60)	1 (10)	3 (30)	10
C34	5 (55.6)	2 (22.2)	2 (22.2)	09
C35	7 (77.8)	2 (22.2)	0 (0)	09
C36	4 (66.6)	1 (16.7)	1 (16.7)	06
C37	1 (20)	2 (40)	2 (40)	05
C38	3 (100)	0 (0)	0 (0)	03
Total	134 (71.6)	22 (11.8)	31 (16.6)	187

[a]Number of *Colletotrichum gloeosporioides Nit* mutants and In parentheses: percentage of mutants.

mutants among 187 mutants; 84.3% of these isolates were identified as *nit*1/*nit*3 and 15.7% as *NitM*. Similar recovery frequencies of *nit1* and *NitM* classes also were found for *C. coccodes* (Nitzan et al., 2006), *Verticillium lecanii* (Korolev and Gindin, 1999) and *V. dahliae* (Korolev and Katan, 1997; Sanei et al., 2008) on WAC medium, and for *C. destructivum*, *C. gloesporioides*, and *C. fragariae* on potato dextrose agar medium amended with chlorate (Brooker et al., 1991). Thus, it appears that, in *C. gloeosporioides*, the genetic control of nitrate assimilation in particular, and nitrogen catabolism in general, are similar to those of other species of *Colletotrichum*, and other genera, such as *Aspergillus*, *Neurospora*, *Fusarium*, and *Verticillium*, whose VCGs have been previously studied. According to Strausbagh (1993), some loci can be mutated more frequently. The frequency of *Nit1* and *NitM* mutants is higher than the *Nit3* mutants. The dominance of *Nit1* has also been reported by several authors (Korolev and Katan, 1997; Lachquer et al., 2002; Tsror and Levin, 2003).

The present study demonstrates for the first time the occurrence of VCG diversity within *C. gloesporioides* in Iran. The comparison of selected Nit mutants produced only four vegetative compatibility groups (VCGs). This shows that *C. gloesporioides* population is homogeneous and that the isolates are genetically closely related. The presence of low vegetative compatibility groups (VCG) in *C. gloesporioides* has been not reported by Beever et al. (1995) identified 11 VCG out of a population of 32 isolates and by Nitzan et al. (2006) identified 7 VCG out of a population of 123 *C. coccodes* isolates. The assignment of the isolates only to four VCGs may indicate a possible adaptation of these VCGs to climate conditions in specific areas. Finding the same VCGs in different countries could be due to founder effect but it could be also due to other reasons, such as gene flow, selection, or limited number of polymorphic vegetative incompatibility genes.

There is a correlation between VCG and pathogenicity in *C. gloesporioides* from the olive isolates on apple fruit. VCG1 isolates were observed as the most aggressive and had the colonization levels but isolates of VCG2 were moderately aggressive. These results are in agreement with results from previous studies on *V. dahliae*, which have demonstrated some correlations between VCGs and virulence on certain hosts (Bhat and

Table 3. Results of crossings on a nitrate minimum medium (MM) between complementary mutants generated from clones of *V. dahliae* isolated from different hosts.

	C1	C2	C3	C6	C8	C11	C13	C15	C18	C19	C20	C23	C24	C26	C27	C28	C29	C30	C31	C33	C34	C35	C36	C37	C38
C1	+	+	-	-	+	+	+	+/-	+/-	+/-	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-
C2		+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-
C3			+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
C6				+	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	+	+/-	+	-
C8					+	+	+	+	+/-	+/-	+/-	+	+	-	+	+	+	-	+	+	+	-	-	-	-
C11						+	+	+/-	+	+	+	+	+	-	+	+	+/-	-	+	+	+/-	-	-	-	-
C13							+	+/-	+	+	+/-	+	+	-	+	+	+	-	+	+	+	-	-	-	-
C15								+	+	+	+	+	+	-	+/-	+	+	-	+	+/-	+	-	-	-	-
C18									+	+	+	+	+	-	+	+	+/-	-	+	+	+	-	-	-	-
C19										+	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-
C20											+	+	+	-	+	+	+	-	+	+	+	-	-	-	-
C23												+	+	-	+	+	+	-	+/-	+	+	-	-	-	-
C24													+	-	+	+	+	-	+	+	+/-	-	-	-	-
C26														+	-	-	-	-	-	-	-	+	+/-	+	-
C27															+	+	+	-	+	+	+	-	-	-	-
C28																+	+	-	+	+	+	-	-	-	-
C29																	+	-	+	+	+	-	-	-	-
C30																		+	-	-	-	-	-	-	+
C31																			+	+	+	-	-	-	-
C33																				+	+	-	-	-	-
C34																					+	-	-	-	-
C35																						+	+	+/-	-
C36																							+	+	-
C37																								+	-
C38																									+

+: Formation of heterokarotic mycelium in the contact zone. +/-: Weak reaction. - : No complementation between the mutants tested.

Subbarao, 1999; Sanei et al., 2008). Similarly, the correlations between VCG and pathogenicity may be significant in *C. coccodes* (Nitzan et al., 2006). Differences in aggressiveness of VCGs may have practical importance, especially for olive trees in which *C. gloesporioides* has become an important pathogen. Data on the VCG distribution of detected populations, and of the relative aggressiveness of each VCG, would enable a more accurate evaluation of potential damage and the necessary control measures. Determination of potential aggressiveness to potato in *C. gloesporioides* populations is also important for accurate selection of isolates when screening resistant and tolerant lines in breeding programs.

Even though the *C. gloesporioides* tested isolates come from different geographical sites, they all belong to the same VCG suggesting the absence of relation between VCG and their geographic origin. This is in accordance with the result of Joaquim and Rowe (1991). The absence of VCG-geographical relation was also observed by Daayf et al. (1995) inside a population of 27 *V. dahliae* strains from Africa, Asia, Europe and the United States. Corell et al. (1988) also reported the absence of such relation in a population of *V. albo-atrum*.

The existence of multiple VCGs amongst strains of *C. gloeosporioides* from the various crops is consistent with the known variability of this species and with previous reports. Brooker et al. (1991) tested single strains of *C. gloeosporioides* from apple, vicia bean, and water primrose and found each to be in a different VCG. A preliminary report, Correll et al. (1991) indicated that isolates of *C. gloeosporioides* from apple, lime and pecan generally belonged to unique VCGs, whereas those from northern jointvetch belonged to a single VCG. Results of Beever et al. (1995) confirm the multiplicity of VCGs amongst apple strains, and indicated multiple VCGs amongst citrus strains.

While no strains from different fruits belonged to the same VCG, too few strains have been examined to conclude that this is a widespread feature of *C. gloeosporioides* from fruit. It is reasonable to conclude that strains in the same VCG are genetically close and thus conspecific, although the reverse is not true. Presuming that there are multiple *vie* loci in *C. gloeosporioides,* sexual crossing between incompatible strains differing at more than one locus will generate progeny with different combinations of *vie* genes, thus leading to multiple VCGs. We suggest that the multiplicity of VCGs amongst the culturally similar apple isolates reflects this process, resulting from occasional crossing between strains. Given the number of VCGs detected in the relatively small number of strains examined, it is likely that study will reveal additional VCGs on this host. Further studies are needed to determine the genetic relationships between the various cultural groups. Some may be best considered distinct species. The present studies indicate that different VCGs exist even within a given cultural group, and thus VCGs will not be a useful tool for defining species limits, at least in those groups where sexual reproduction occurs. Our data for a single tree indicate that at least three different primary infection sources were present. The observation that lesions on the one fruit were in one VCG is consistent with the secondary spread of infections around that fruit.

Conclusion

Four multimember VCGs observed within the species *C. gloesporioides* indicate population differentiation and genetic diversity within subpopulations. The aggressiveness of VCG3 isolates to potato was the highest. We have defined a set of 12 tester strains for conducting VCG complementation tests that can now be used to characterize and begin to compare populations of *C. gloesporioides* worldwide. However, in order to improve understanding of the structure of populations, additional isolates from other potato-growing regions should be tested for VCGs. Moreover, isolates from other locations in addition to Europe and Israel must be included in order to establish international tester strains.

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