

## SHORT COMMUNICATION

GENETIC DIVERSITY OF *FUSARIUM VERTICILLIOIDES* ISOLATES FROM MAIZE IN IRAN BASED ON VEGETATIVE COMPATIBILITY GROUPING

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## SUMMARY

Genetic diversity among *Fusarium verticillioides* isolates was analyzed using vegetative compatibility groups (VCGs). Forty-four isolates of *F. verticillioides* from stalks and seeds were recovered in maize-producing areas in Iran during 2004-2005. Chlorate-resistant nitrate non-utilizing (*nit*) mutants were recovered from selected isolates of *F. verticillioides* and used in complementation (heterokaryon) tests. Vegetative compatibility tests by pairing *nit* mutants identified 25 VCGs among 44 isolates. Twenty-one isolates belonged to single-member VCGs and the remaining 23 isolates belonged to four multimember VCGs. VCG1 was the largest group and included 17 isolates, whereas the other three multimember VCGs contained two isolates each. It is concluded that natural populations of *F. verticillioides* in Iran are genetically highly divergent and include isolates representing a potential risk for disease development.

*Key words:* *nit* mutants, VCG, *Zea mays*, stalk rot, ear rot.

*Fusarium verticillioides* (Sacc.) Nirenberg [*Gibberella fujikuroi* (Sawada) Wollenw., mating population A], a prevalent pathogen of agriculturally important crops (Leslie, 1991; 1995), is associated with stalk and ear rot on maize in Iran. In 2004 and 2005, a high disease incidence was observed in some maize fields. Most recent assessments of fungal pathogens have used multilocus markers to detect populations (Milgroom, 1996). Vegetative compatibility groups (VCGs) have been used as a marker for genotyping field populations of *Fusarium* (Farrokhi-Nejad and Leslie, 1990; Campbell *et al.*, 1992; Leslie, 1993). Members of the same VCG can form a stable heterokaryon, and share an identical set of alleles at about 10 *vic* loci (Leslie, 1993). Diversity for VCGs was very high in *F. verticillioides* isolates from different regions of Argentina and Costa Rica, where 28 and 34 different VCGs were found among 36 and 39

isolates, respectively (Danielsen *et al.*, 1998; Chulze *et al.*, 2000). A comprehensive Iranian *F. verticillioides* population had never been analyzed before.

In this study, we analysed the genetic diversity of 44 *F. verticillioides* isolates collected during two successive years in Iran, prompted by the increasing economic impact of this pathogen on maize. Understanding the genetic structure of a population reflects its evolutionary history and its potential to evolve. Therefore, knowledge of the genetic structure of the *F. verticillioides* population might be useful in order to establish effective strategies for controlling the disease (McDonald, 2004). The hypotheses of the current study were that there is VCG diversity within *F. verticillioides* subpopulations and that *G. moniliformis* is capable of sexual reproduction. Our objective was to use VCGs identification to determine the genetic diversity among *F. verticillioides* isolates originating from maize in Iran.

Isolates of *Fusarium* spp were recovered from stalks and seeds from 80 maize fields in 15 locations of 12 Iranian provinces (Ardabil, Esfahan, Tehran, Khuzestan, Fars, Ghazvin, Kermanshah, Golestan, Mazandaran, Hamedan, Kerman and Lorestan) in the maize producing areas, during the 2004-2005 growing season. Three maize seeds and stalk tissue from each sample (one sample per province) were collected and were plated on a medium containing pentachloronitrobenzene (Nash and Snyder, 1962), obtaining 176 isolates of *Fusarium* section *Liseola*, among which *F. verticillioides* was the most frequent (73% of the population), followed by *F. proliferatum* (27%).

Single spores were obtained by dilution plating and identified on the morphological criteria of Leslie and Summerell (2006). For this purpose, subcultures were made on SNA (Spezieller Nährstoffarmer Agar), CLA (Carnation Leaf Agar) and PDA (Potato dextrose Agar), incubated for 7 days at 25° C (Fisher *et al.*, 1982; Gerlach and Nirenberg, 1982). Forty-four isolates were selected from a total of 129 isolates of *F. verticillioides* for VCG determination, which was based on complementation reaction between NitM (or *nit3*) and *nit1* mutants on minimal medium. Pairings to test for vegetative compatibility were made in 24-well hybridoma plates as described by Klittich and Leslie (1988b). Pairs of isolates that exhibit-

ed vigorous aerial growth at the contact zone of the two *nit* mutant, indicating the formation of a heterokaryon, were determined as vegetatively compatible and were assigned to the same VCG. Otherwise, isolates were defined as belonging to different VCGs.

Nitrate non-utilizing (*nit*) mutants were recovered from the 44 isolates of *F. verticillioides* and used in complementation tests to identify isolates belonging to the

same VCG. Recovery of *nit* mutants was done according to the methods described by Klittich and Leslie (1988a).

Mycelial discs from each isolate were placed on minimal medium plus 3% KClO<sub>3</sub> for mutant selection. Inoculated plates were incubated at 24-25°C and were examined weekly for the appearance of fast growing sectors. The physiological phenotypes of *nit* mutants were assigned to *nit1*, *nit3*, and NitM classes based on differen-

**Table 1.** Characterization of *nit* mutants from *F. verticillioides* isolated from maize grown in different locations of Iran.

Isolate (No.) <sup>a</sup>	Location	Year of isolation	Infected tissue	VCG group <sup>b</sup>
1	Moghan	2005	ear	1
2	Moghan	2005	ear	5
3	Moghan	2005	ear	6
4	Esfahan	2005	ear	2
5	Esfahan	2005	ear	1
6	Esfahan	2005	ear	1
7	Karaj	2005	ear	7
8	Karaj	2005	ear	1
9	Varamin	2005	ear	8
10	Varamin	2005	ear	1
11	Shoosh	2005	ear	9
12	Shoosh	2005	ear	10
13	Dezful	2005	ear	11
14	Dezful	2005	ear	12
15	Zareghan	2005	ear	1
16	Zareghan	2005	ear	13
17	Marvdasht	2005	ear	14
18	Marvdasht	2005	ear	15
19	Ghazvin	2004	ear	16
20	Boin-Zahra	2005	ear	2
21a	Paveh	2005	ear	1
21b	Paveh	2005	ear	17
22	Paveh	2005	ear	1
23	Karand	2005	ear	4
24a	Karand	2005	ear	3
24b	Karand	2005	ear	4
25a	Kermanshah	2005	ear	1
25b	Kermanshah	2005	ear	18
26	Harsin	2005	ear	1
27	Gorgan	2005	ear	19
28	Gorgan	2005	ear	20
29	Gorgan	2005	ear	21
30	Sari	2005	ear	22
31	Sari	2005	ear	23
32	Sari	2005	ear	1
33	Asad-Abad	2005	ear	1
34	Asad-Abad	2005	ear	1
35	Asad-Abad	2005	ear	1
36	Asad-Abad	2005	ear	1
37	Asad-Abad	2005	ear	24
38	Brojerd	2005	stalk	3
39	Baft	2004	stalk	1
40	Baft	2004	stalk	1
41	Baft	2004	stalk	25

<sup>a</sup>Isolates with identical number but different letters (e.g.21a and 21b) were obtained from the same ear sample.

<sup>b</sup>VCG = vegetative compatibility group.

tial growth on basal medium with NaNO<sub>3</sub>, NaNO<sub>2</sub>, hypoxanthine, ammonium tartrate and uric acid as sole nitrogen sources.

VCG assignment was based on complementation reaction between NitM (or *nit3*) and *nit1* mutants on minimal medium. Pairings to test for vegetative compatibility were made in 24-well hybridoma plates as previously described by Klittich and Leslie (1988b). Pairs of isolates that exhibited vigorous aerial growth at the contact zone of the two *nit* mutant, indicating the formation of a heterokaryon, were determined as vegetative compatible and were assigned to the same VCG. Otherwise, isolates were defined as belonging to different VCGs.

All 44 *F. verticillioides* isolates spontaneously produced chlorate-resistant sectors on MMC and grew on MM as thin mycelium with no aerial mycelium (*nit* mutants) because of their inability to utilize nitrate as a sole nitrogen source. Among the *nit* mutants, the *nit1* class composed 78% of the total number of mutants, while NitM mutants represented 4% and 18% were *nit3* mutants.

Based on positive complementation reactions with the complementary *nit* mutants, we identified four multi-member VCGs among the 44 isolates. Twenty-one isolates (46%) could not be assigned to any of these four VCGs. All isolates for which we recovered both *nit1* and NitM mutants demonstrated self-compatibility. VCG1 was the largest and the most frequent group in Iran, including 38% of all isolates (Kermanshah 9%, Hamedan 9%, Tehran 5%, Esfahan 4.5%, Kerman 4.5%, Mazandaran 2%, Fars 2%, Ardabil 2%); VCG2, VCG3, and VCG4 included 16% of all isolates (Table 1).

This study has shown that *F. verticillioides* is the most frequent *Fusarium* species on maize in Iran. Segregation of *F. verticillioides* isolates obtained from maize into VCGs in Iran is reported for the first time. We identified 25 VCGs among 44 isolates, accounting for a genetic diversity (number of VCGs/number of isolates) of 0.57. The analyzed population of *F. verticillioides* is genotypically diverse. Twenty-one VCGs had only one member, and the remaining 23 belonged to four multi-member VCGs. VCG1 was the largest and the most frequent group in Iran and included 17 isolates, whereas the other three multimember VCGs had two members each. Isolates belonging to VCG1 were collected from 8 provinces, indicating that genetic variation across geographic locations also occurs in Iran, thus confirming evidence obtained in previous observations (Danielsen *et al.*, 1998; Chulze *et al.*, 2000). Six isolates from the same ear samples were ascribed to different VCGs, which indicates that high genotypic diversity occurs in *F. verticillioides* from the same ear. These results are in agreement with results from previous studies on *F. verticillioides*, demonstrating that the population of this fungus is genotypically highly diverse (Danielsen *et al.*, 1998; Chulze *et al.*, 2000).

Since parasexuality is dependent upon the formation

of a heterokaryon, parasexual recombination occurs only between members of the same VCG (Leslie, 1993). Forty-four *F. verticillioides* isolates belonged to 25 VCGs, accounting for a genotype diversity estimate of 0.57.

Preliminary results on sexual fertility indicate that sexual reproduction does not occur frequently in the population analyzed (Mirzadi Gohari *et al.*, unpublished information). Since clonal spread by rainsplash or wind over large distances is unlikely, the same VCG genotype was probably introduced into different sites as seed-borne inoculum. Infected maize seed must be a significant vehicle for the dissemination of pathogen genotypes throughout Iran. In addition, we think that fungal populations including VCG1 may be adapted to a maize genotype which was commonly cultivated in 2004-2005. However, in order to improve understanding of the structure of populations, additional isolates from other maize-growing regions should be tested for VCGs. Moreover, neutral DNA markers such as RAPDs and AFLP (McDonald and McDermott, 1993) would generate further results on the genetic structure of *F. verticillioides* populations in maize.

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