Journal of Agricultural Technology 2011 Vol. 7(1): 143-148
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

Investigation on genetic diversity of *Fusarium verticillioides* isolated from corn using vegetative compatibility groups and relation of VCGs to the pathogenecity

Mohammadi, A.[1*] and Mofrad, N.N.[2]

[1]Department of Plant Pathology, Faculty of Agriculture, Birjand University, Birjand, Iran
[2]Faculty of Agriculture, Islamic Azad University of Birjand, Birjand, Iran

Mohammadi, A. and Mofrad, N.N. (2011). Investigation on genetic diversity of Fusarium verticillioides isolated from corn using vegetative compatibility groups and relation of VCGs to the pathogenecity. Journal of Agricultural Technology 7(1): 143-148.

Forty isolates of *Fusarium verticillioides* were recovered from corn seeds using Nash & Snyder media. Seed samples had been collected from the major producing area in Khuzestan and Ardabil provinces. Isolates were tested for their pathogenecity on the corn-detached stalks. Results revealed that all isolates were pathogenic to the plant samples tested. Four hundred and twenty nitrate non-utilizing mutants were generated from corn seeds isolates. Media used for generation nit mutants were potato dextrose agar (PDA), minimal & Czapeck agar amended with 3% chlorate potassium. Nit mutants were divided into three phenotypic classes (nit1, nit3 and nitM) based on their growth on the medium containing different nitrogen sources. For tested isolates, 265, 95 and 95 nit1, nit3 and nitM were generated respectively. Nit mutants were used to force heterokaryon formation to determine distribution of VCGs and their relation to pathogenecity and geographic origin. For corn seeds, isolates, 39 VCGs were determined. Of these, 38 were single and one was single member VCGs. Specific relation was not observed between VCGs and geographic origin of the isolates in this study. It means that genetic diversity among population of *F. verticillioides* is very high.

Key word: *Fusarium verticillioides,* moniliforme, mutant, nit, vegetative compatibility

Introduction

Fusarium verticillioides (*Fusarium moniliforme*) has been associated with human and animal toxicoses since it was first described in 1881 (Saccardo, 1881). This species and other anamorphs of *Gibberella fujikuroi* are the fungi most commonly associated with maize production in Iran and other temperate regions of the world (White, 1999). Fusarium species are capable of causing seedling disease, root rots, stalk rots and ear rots of maize as well as damaging stored grain. Although yield usually is not much affected, kernel infection by *G. fujikuroi* is of

* Corresponding author: Mohammadi, A.; e-mail: Amohammadi@birjand.ac.ir

concern because of the loss of grain and seed quality and the potential occurrence of fumonisins and other mycotoxins (Munkvold and Desjardins, 1997). This toxin has been shown to be toxic and carcinogenic to a variety of experimental animals including baboons, chickens, ducklings, mice, rabbits, and rats (Pedersen and Miller, 1999) and to cause pulmonary edema in swine and equine leukoencephalomalacia (Nelson *et al.*, 1993). Consumption of contaminated grain is correlated with human esophageal cancer risk in Transkei, South Africa and in the People's Republic of China (Zhen, 1984). Corn is one the most important human and animal food in Iran. The objective of this study is isolation of *F. verticilioides* from corn seed in the provinces of Khuzestan and Ardabil and identification of its genetic diversity by vegetative compatibility groups.

Materials and methods

Strains

Fusarium moulds in maize seeds were determined by plating seeds on 15 ml of sterile in Petri plates. The seeds were surface-sanitized with full strength household bleach (5.25% NaOCl) for 1 min, rinsed with sterile distilled water three times, and dried on sterile paper towels. Nash & Snyder medium (NSM) was used as a selective medium for isolation of *Fusarium* species. This media allow formation of large, easily recognizable colonies (Chen *et al.*, 2007). In addition, the growth of other moulds such as *Alternaria*, *Epicoccum*, *Penicillium* and *Mucoraceous* species is restricted on NSM. Percent infection was determined by counting the number of seeds from which internal mould contaminants grow (Leslie and Summerell, 2006). Fusarium colonies were observed microscopically. Those colonies identified as *F. verticilioides* were transferred to carnation-leaf agar (CLA) and potato dextrose agar (PDA). Single spore isolation was made from each colony and isolates were identified morphologically to species based on characteristics of the macroconidia, phialides, microconidia, chlamydospores, and colony growth traits (Leslie and Summerell, 2006).

Pathogenecity test

A cut in the stem or root was made using a sterilized knife. A 1x2 cm inoculums block from 5 days old culture of a test fungus on PDA was placed in the gap and the inoculated portion was wrapped with Para film. A 1x2 cm PSA block without fungus was placed in the control plants. The wrapping material was removed from the stems after 2 weeks of inoculation. Plants were

monitored for the development of disease symptoms and isolation was made from stem to confirm the pathogenicity.

Vegetative compatibility

VCGs were determined using complementation of nitrate no utilizing (*nit*) mutants as a visual indicator of heterokaryon formation (Jo *et al.*, 2008). *Nit* mutants were generated from each of the 40 *F. verticillioides* isolates on PDA containing 3% KClO3. The concentration of KClO3 was increased to 5% for isolates that were not restricted by 3% KClO3 (Klittich and Leslie, 1988). The fast growing, chlorate-resistant sectors originating from the initially restricted colony, which grew thinly but expansively on Puhalla's minimal medium (MM), were considered *nit* mutants(Puhalla, 1985). *Nit* mutants were phenotypically classified by their growth on basal medium (MM without NaNO3) amended with one of several nitrogen sources (Pasquali *et al.*, 2005). *Nit* mutants (*nit1, nit3*, and NitM) generated from each of the *F. verticillioides* isolates were paired with tester strains (*nit1* and NitM) of each of the established VCGs Pairings were made on MM in 9-cm Petri dishes incubated at temperature 25°C in the dark and scored for complementation 7 and 14 days later. When a mutant successfully formed a complementary heterokaryon with a given tester, its parent was placed in the corresponding VCG (Aqeel *et al.*, 2008).

Results and discussion

Forty isolates of *F. verticillioides* were recovered from corn seed from Moghan and Khuzestan province. All samples from corn seeds were infected with f. verticillioides. Inoculated stem showed discoloration from red to brown, all isolates have pathogenecity, but no changes were observed in control. *F. verticillioides* isolates produced chlorate-resistant sectors on MM complemented with chlorate. The most number of sectors were observed at 3.0% (w/v) of chlorate concentration, followed by at 2.5%, 3.5%, and 4.0%. There were also great differences in sectoring frequency of each isolate. The majority of the chlorate-resistant sectors recovered was unable to utilize nitrate as a sole nitrogen source and consequently grew as thin expansive colonies without aerial mycelium on MM. A few chlorate-resistant sectors were able to utilize nitrate. No chlorate-resistant sector was observed in PDA complemented with chlorate. The phenotypes of 420 *nit* mutants from *F. verticillioides* were determined by their colony morphology on media containing nitrate, nitrite, hypoxanthine, uric acid, or ammonium tartrate as a sole nitrogen source. The *nit* mutants could be divided into three classes; *nit1* (a mutation of nitrate reductase

structural locus), *nit3* (a mutation of nitrate-assimilation pathway specific locus), and NitM (mutations that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity). The majority of *nit* mutants was *nit1* (63/10%), and followed by *nit3* (22/61%) and NitM (14/29%). Physiological complementation among *nit* mutants with different mutations was indicated by the development of dense aerial mycelia where the mycelia of the *nit* mutants came in contact and anastomosed to form a heterokaryon. Thirty-nine VCGs were determined in tested isolates, one group with two isolates and 38 VCGs with one isolate. There was no correlation between geographical distribution, pathogenecity and VCG groups. *F. verticillioides* occurs in all parts of growing corn plant throughout the growing season. This fungus is universally present in seed, and is inactive in stalk tissues until the plant approaches maturity or is injured. *F. Verticillioides* might over winter as mycelia on infected corn stalks and in the soil (Munkvold and Desjardins, 1997). Results of this research showed infection of Iranian corn seeds by F. verticillioides. Spores of this pathogen were distributed by wind between corn fields (Ooka and Kommedahl, 1977). Long distance distribution was made up by seed infection. *F. verticillioides* is capable of causing seedling disease, root rot, stem rot or ear rot of maize or infection may be symptomless. Although yield usually is not much affected, kernel infection by *Fusarium* is of concern because of the loss of grain and seed quality and the potential occurrence of fumonisins and other mycotoxins (Munkvold and Desjardins, 1997).The frequency of resistant sectoring on MMC was different when different concentrations of chlorate were supplemented. The 3.0% of chlorate produced the highest frequency of sectoring in this study. Sectoring frequency of *F. verticilioides* has been shown to be heritable and to vary among isolates (Klittich and Leslie, 1988). The wide range of sectoring frequency in plant pathogenic fungi on different concentrations of chlorate has also been suggested as a selective advantage for rapid adaptation to environmental stresses such as fungicides and host resistance (Pasquali *et al.*, 2005). No chlorate-resistant sector was observed in PDA complemented with chlorate. However, similar frequency of sectoring was observed in both PDA and MM complemented with chlorate in other *Fusarium* spp. (Correll *et al.*, 1987). In *F. verticilioides*, heterokaryon self-incompatibility is heritable trait and may be controlled by a single nuclear gene (Leslie, 1993). Isolates carrying mutations that prevent them from fusing to form heterokaryons, even with themselves, have been identified in field population of *Fusarium oxysporum* (Bosland and Williams, 1987), *F. verticilioides* (Klittich and Leslie, 1988), and *F. subglutinans* (Correll *et al.*, 1992). VCG groups showed high genetic diversity between Iranian isolates of *F. verticilioides*. Specific relation was not observed between VCGs

and geographic origin of the isolates in this study. It means that genetic diversity among population of *F. verticilioides* is very high and these populations are genetically quite distinct.

References

Aqeel, A.M., Pasche, J.S. and Gudmestad, N.C. (2008). Variability in Morphology and Aggressiveness Among North American Vegetative Compatibility Groups of *Colletotrichum coccodes*. Phytopathology. 98: 901-909.

Bosland, P.W. and Williams, P.H. (1987). An evaluation of *Fusarium oxysporum* from crucifers based on pathogenecity, isozyme polymorphism, vegetative compatibility, and geographic origin. Can. J. Bot. 65:2067-2073.

Chen, Y., Wang, J.X., Zhou, M.G. Chen, C.J. and Yuan, S.K. (2007). Vegetative Compatibility of *Fusarium graminearum* Isolates and Genetic Study on Their Carbendazim-Resistance Recombination in China. Phytopathology. 97: 1584-1589.

Correll, J.C., Gordon, T.R. and McCain, A.H. (1992). Genetic diversity in California and Florida populations of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. Phytopathology. 82:415-420.

Correll, J.C., Klittich, C.J.R. and Leslie, J.F. (1987). Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology. 77: 1640-1646.

Jo, Y.K., Chang S.W., Rees, J. and Jung, G. (2008). Reassessment of Vegetative Compatibility of *Sclerotinia homoeocarpa* Using Nitrate-Nonutilizing Mutants. Phytopathology. 98: 108-114.

Klittich, C.R.J. and Leslie, J.F. (1988). Nitrate reduction mutants of *Fusarium moniliforme (Gibberella fujikuroi)*. Genetics. 118:417–423.

Leslie, J.F. (1993). Fungal vegetative compatibility, Annu. Rev. Phytopathol. 31:127-150.

Leslie, J.F. and Summerell, B.A. (2006). The Fusarium Laboratory Manual. Blackwell Publishing Ltd, UK.

Munkvold, G.P. and Desjardins, A.E. (1997). Fumonisins in maize, can we reduce their occurrence? Plant Dis. 81:556-565.

Nelson, P.E., Desjardins, A.E. and Plattner, R.D. (1993). Fumonisins, mycotoxins produced by Fusarium species: biology, chemistry, and significance.Annu. Rev. Phytopathol. 31:233–252.

Ooka, J.J. and Kommedahl, T. (1977). Wind and rain dispersal of *Fusarium moniliforme* in corn fields. Phytopathology. 67: 1023-1026.

Pasquali, M., Dematheis, F., Gilardi, G., Gullino, M.L. and Garibaldi, A. (2005). Vegetative Compatibility Groups of *Fusarium oxysporum* f. sp. *lactucae* from Lettuce. Plant Dis. 89: 237-240.

Pedersen, P.B. and Miller, J.D. (1999). The fungal metabolite culmorin and related compounds. Natural Toxins. 7:305-309.

Puhalla, J.E. (1985). Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.

Saccardo, A. (1881). Fungi Italici AutographiceD elinati, Figure 879. Patavii.
White, D.G. (1999). Compendium of Corn Diseases. 3rd ed. The American Phytopathological Society, St. Paul, MN.
Zhen, Y.Z. (1984). Isolation and culture of fungi from the cereals in five high- and three low-incidence counties of esophageal cancer in Henan province (China). Zhonghua Zhongliu Zazhi 627-29.

(Received 2 Febeuary 2010; accepted 29 October 2010)