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Short communication

Detection of genetic variation among Indian wheat head scab pathogens (*Fusarium* spp./isolates) with microsatellite markers

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Abstract

Fusarium head blight (FHB) of wheat is responsible for extensive damage of wheat in humid and semi-humid regions of the world. Presently, FHB of wheat is a minor disease in India but due to global climate change, there is a chance that moist conditions and high humidity resulting from more rainfall during mid-anthesis could increase the susceptibility of wheat to *Fusarium* infection. For the present study, 27 isolates of three *Fusarium* spp. viz., *Fusarium graminearum*, *Fusarium verticillioides* and *Fusarium oxysporum* were isolated from naturally infected wheat sampled from Punjab, Himachal Pradesh and Wellington (Tamil Nadu) during 2000–2003. Genomic DNA was isolated from fresh mycelia using the CTAB method. *Fusarium* spp./isolates were analyzed with four newly developed microsatellite markers (MS-Fg1353, MS-Fg6808, MS-Fg307 and MS-Fg3654) and six previously published microsatellite markers (MS-Fg97, MS-Fc1, MS-Fg103, MS-Fg30, MS-Fg75 and MS-Fg90). All markers amplified a DNA fragment of variable length for different *Fusarium* spp./isolates. Microsatellite markers, MS-Fg103, MS-Fg103 did not amplify *F. oxysporum* and *F. verticillioides* isolates, respectively. MS-Fg307 amplified a fragment of 200 bp with *F. graminearum* isolates of Wellington. This study has shown that there is considerable genotypic variability among *Fusarium* spp./isolates causing FHB of wheat in India.

Keywords: *Fusarium* spp.; Isolates; Head scab; Microsatellites; Genetic variability

1. Introduction

Fusarium head blight (FHB) or head scab of wheat is a global problem with recent outbreaks reported in Canada, Europe, Asia, Australia and South America (McMullen et al., 1997). A high throughput screening of a large number of samples from Netherlands demonstrated that *Fusarium graminearum* was the most abundant species in the *Fusarium* complex during 2000 and 2001 (Cees Waalwijk et al., 2003). *Fusarium* head blight is mainly caused by *F. graminearum*, *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium poae* and *Fusarium equiseti* (Bottalico and Perrone, 2002). In addition to causing yield losses, *Fusarium* spp. can produce mycotoxins such as trichothecenes, which are highly toxic to plants, animals and humans (Desjardins et al., 1993; Bennett and Klich, 2003). Strains of *F. graminearum* are known to produce type B trichothecene deoxynivalenol (DON), which is a potential inhibitor of eukaryotic protein synthesis (Rotter et al., 1996; Bottalico and Perrone, 2002). Currently, FHB is of minor importance to India but can cause significant yield loss if rain occurs during mid-anthesis in the foot hills of Punjab, Himachal Pradesh and Tamil Nadu. Changes in tillage practices, principally the move toward conservation tillage and reduced-till systems, contributed to the recent FHB epidemics in Upper Midwest of the United States of America (Dill-Macky and Jones, 2000). The *Fusarium* diseases, which have already established in the Indian subcontinent and are likely to increase under the impact of global warming and the fast spread of reduced tillage practices in the main wheat belt, the north west plains of

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India. During 2005, due to continuous rain in March in Punjab, severe head scab appeared on durum cultivar PDW 274 in the Gurdaspur area of Punjab (Saharan et al., 2007). In India, three *Fusarium* spp. viz., *F. graminearum* teleomorph *Gibberella zeae* (Schwein) Petch, *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *Fusarium moniliforme* J. Sheld) and *Fusarium oxysporum* Schlecht are mainly responsible for FHB (Saharan et al., 2003). *F. graminearum* causing scab of wheat was first reported from Arunachal Pradesh (Roy, 1974) and from Wellington, Nilgiri Hills, Tamil Nadu (Brahma and Singh, 1985; Saharan et al., 2003).

Most wheat cultivars currently grown in India are susceptible to FHB. Control of the disease has been difficult, because of the complex nature of the host/pathogen/environment interaction. Promising options for controlling FHB include chemical measures and the development of resistant cultivars. Fungicides like tebuconazole, prochloraz and bromuconazole were effective for controlling FHB and reducing DON concentration by 43% (Menniti et al., 2003). Some other fungicides also successfully controlled FHB (Matthies and Buchenauer, 2000; Siranidou and Buchenauer, 2001; Haidukowski et al., 2004; Beyer et al., 2006), but food safety concerns and inconsistent results due to the complexity of causal organisms, timing of application and development of fungicide resistance in the pathogen population, limits the chemical management option (Jones, 2000). Thus, growing of wheat cultivars resistant to *Fusarium* spp. has been accepted as the most reliable, economic, environment-friendly and effective method of managing this disease. However, for applying efficient strategies in the breeding process, knowledge about the genetic diversity and structure of naturally occurring pathogen populations are indispensable. Understanding the genetic structure of pathogen populations may also provide insights into the epidemiology and evolutionary potential of *Fusarium* spp. and could lead to improved management strategies. Species of *Fusarium* can tolerate diverse environmental conditions and have high levels of intraspecific genetic and genotypic diversity (Miedaner et al., 2001). Studies on molecular variation in *Fusarium* spp. are numerous. Like in other pathogen systems, molecular techniques have become reliable and highly suitable tools for identifying *Fusarium* species and for assessing genetic variation within collections and population (Burgess et al., 1996).

A novel method for detection of genetic variation among *Fusarium* spp./isolates used in the present study is based on PCR of a microsatellite. Microsatellites are loci composed of simple sequence repeats, which are randomly distributed throughout the genome of fungi and other eukaryotes (Li et al., 2002; Wostemeyer and Kreibich, 2002). For the development of microsatellite markers, sequences with simple sequence repeats must be identified in a genome database or a repeat-enriched clone library. Many microsatellites contain a variable number of repeats in different individuals. This polymorphism is detected as length polymorphism of a PCR product generated with oligonucleotide primers designed on conserved flanking regions. Polymorphic microsatellites have been used as genetic markers for assessing the genetic variation in populations of fungal plant pathogens (Sexton and Howlett, 2004; Tenzer et al., 1999). Few microsatellite markers are known for *F. graminearum* (Giraud et al., 2002; Suga et al., 2004; Naef and Défago, 2006). The current knowledge on genotypic variations among isolates of *Fusarium* spp. obtained from different geographic regions of India is very limited. The objective of the present study was to characterize genetic variability within and among isolates of different *Fusarium* spp. belonging to three different sections viz., *F. graminearum* (section Discolor), *F. oxysporum* (section Elegans), *F. verticillioides* (section Liseola) using a set of newly developed and established microsatellites.

2. Materials and methods

2.1. Collection of diseased samples and isolation of *Fusarium spp.*

Wheat ears with visual symptoms of head scab were sampled from different fields in the high Himalayas of Himachal Pradesh (4000 masl), Punjab (300–400 masl) and Wellington (Nilgiri Hills, Tamil Nadu, 1850 masl) during 2000–2003 (Table 1). Wheat kernels were surface disinfected by washing them with 0.5% sodium hypochlorite for 1 min. Subsequently, the kernels were washed extensively with sterile distilled water and were plated on potato dextrose agar (PDA) media. All isolates were generated from monospores and maintained on PDA. Identification of *Fusarium* spp. was confirmed by mycologists from Indian Type Culture Collection (ITCC), Department of Plant Pathology, Indian Agricultural Research Institute, New Delhi. Lyophilized mycelia (250 mg) of 27 isolates of different *Fusarium* spp. viz., *F. graminearum*, *F. verticillioides* and *F. oxysporum*, which showed pathogenic variability were used in the present study.

2.2. Development of new microsatellites for DNA amplification of Fusarium spp./isolates

Fungal DNA was isolated using the CTAB method (Murray and Thompson, 1990). Four new microsatellite markers (MS-Fg1353, MS-Fg6808, MS-Fg307, MS-Fg3654) were developed as described by Naef and Défago (2006). SSR-containing sequences were found by screening the genome sequence data base (Syngenta, Torrey Mesa Research Institute, San Diego, CA, USA) as described in Naef et al. (2006). Oligonucleotide primers for PCR amplification were designed on the flanking regions of repeats with the online service "Primer3" (Rozen and Skaletskyand, 2000). Specificity and length polymorphism were tested with DNA of closely related and other organisms.