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Short communication

Diversity analysis of *Fusarium oxysporum* f. sp. *gladioli* isolates using Fatty Acid Methyl Ester (FAME) profiling

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ABSTRACT

Two geographically different isolates of *Fusarium oxysporum* f. sp. *gladioli*, namely Isolate-1 collected as infected gladiolus corms from research farm of IARI, New Delhi, and Isolate-2 from infected corms at research farm of GBPUA&T, Pantnagar, Uttarakhand were selected for the experiment. The fatty acid methyl ester profile was determined by initially converting the cellular lipids to their corresponding Fatty Acid Methyl Esters (FAMEs). Both the *Fog* isolates were quite distinct with respect to the fatty acids profiles and also percentage compositions of different fractions, which ranged from less than 1 to over 46% of the total identified fatty acids content. The most common and abundant fatty acids in the two *Fusarium* isolates were palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), and arachidic acid (C20:0) as unsaturated and palmitoleic acid (C16:1 n_7), cis-10-heptadecenoic acid (C17:1 n_8), oleic acid (C18:1 n_9) and linoleic acid (C18:2 n_6), which accounted for 94.45 and 98.35% of the fatty acid profiles in IARI and Pantnagar *F. oxysporum* f. sp. *gladioli* isolates, respectively suggesting that they are distinct and also differ in pathogenecity. This is the first ever attempt to characterize *Fusarium oxysporum* f. sp. *gladioli* isolates using FAME in the country.

Key words: Genetic diversity, Fatty Acid Methyl Esters, *Fusarium oxysporum* f. sp. *gladioli*, isolates.

The microorganisms, such as bacteria, yeasts, moulds, fungi and microalgae accumulate over 25% of their biomass as microbial oil are generally termed as oleaginous due to similarity of their fatty acid composition to that of vegetable oils (Nelson, 5). Most of the fatty acid fractions in the fungal mycelia are palmitic acid, stearic acid, oleic acid and linoleic acid, while other fractions are present, which help in discriminating the different species and biotypes. These phytopathogenic fungi can be reliably and accurately characterized and identified through the fraction examination of the fatty acid composition (Johnk and Jones, 3). The available literature reveal that fatty acid composition has not been studied in detail for comparing and differentiating *Fusarium* fungi causing rots or wilts in different field and horticultural crops. The taxonomy of *Fusarium* spp. is confusing and various classification systems have been proposed (Nelson, 5). Fungal species identification based on morphological traits is problematic since traits like mycelial pigmentation, formation; shape and size of conidia *etc*. are unstable and highly dependent on composition of media and environmental conditions. Furthermore, phenotypic variations are quite abundant and high degree of expertise is required to distinguish closely related *Fusarium* species and to recognize genetic variation within close species (Nelson *et al*., 6). Chemotaxonomy in fungal taxonomy is restricted to comparison of fatty acids, proteins, carbohydrates and secondary metabolites, besides DNA sequences.

The fatty acid composition from *Fusarium oxysporum* B1, a fungal epiphyte from algal crusts, was determined. The major fatty acids estimated in the mycelium were 18:1 omega 9c, 16:0 and 18:0. These compounds amounted to 47% of total fatty acids, whereas remaining identified, were beta-hydroxylated, iso-branched, diunsaturated and odd-numbered derivatives in minor amounts (Ellis *et al*., 1). Matsumoto (4) investigated the efficacy of the two fatty acid analysis protocols (normal (MIDI) and a modified MIDI methods. Modified MIDI method allowed a clear differentiation between *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis lycopersici*. Evaluation of the FAME profiles based on cluster analysis and principal-component analysis revealed that FAME profiles from the tested isolates were correlated with the same vegetative compatibility groups (VCGs) compared to the same races in *F. oxysporum* and thus found useful for characterizing isolates and forma species. Zain (13) studied the amino acid, fatty acid and secondary metabolite profiles of 11 *Fusarium* species. Numerous amino acids including phosphoserine, labrine, glycine, tyrosine, phenylalanine, carnosine, and arginine were detected in all the *Fusarium* species. While aspartic acid, citrulline, valine, cystine, methionine,

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leucine, histidine and lysine were detected in some *Fusarium* species. Similarly, Stępień *et al.* (9) studied the *Fusarium* species variability among the genotypes isolated from pineapple fruits. Forty-four isolates of ten *Fusarium* sp. were obtained from pineapple fruit samples: *F. ananatum*, *F. concentricum*, *F. fujikuroi*, *F. guttiforme*, *F. incarnatum*, *F. oxysporum*, *F. polyphialidicum*, *F. proliferatum*, *F. temperatum* and *F. verticillioides*. *FUM1* and *FUM8* genes were identified in *F. fujikuroi*, *F. proliferatum*, *F. temperatum* and *F. verticillioides*. Cyclic peptide synthase gene (*esyn1* homologue) from the BEA biosynthetic pathway was identified in 40 isolates of eight species. Based on the gene-specific polymerase chain reaction (PCR) assays, none of the isolates tested were found to be able to produce trichothecenes or zearalenone. Later, Shahnazi *et al.* (8) studied the biochemical relationships between *Fusarium solani* and *F. proliferatum* isolates. Cellular fatty acid composition showed that palmitic acid, stearic acid, oleic acid and linoleic acid were the most abundant and accounted for 93.88 and 94.02% of the fatty acid profiles in *F. solani* and *F. proliferatum*, respectively. The most predominant fatty acids were linoleic acid (37.44%) in *F. solani* and oleic acid (39.81%) in *F. proliferatum*. The two species and their isolates significantly differ for most of the individual fatty acids.

In the present study, two geographically different isolates of *Fusarium oxysporum* f. sp. *gladioli*, a). Isolate-1 from infected gladiolus corms from farm of IARI, New Delhi, and b). Isolate-2 from infected corms from GBPUA&T, Pantnagar farm were selected for the experiment. The fatty acid methyl ester profile was determined by initially converting the cellular lipids to their corresponding FAMEs. Thereafter, the gas chromatography was undertaken to differentiate the fatty acid fractions. The profiles obtained were then used for quantification of the different fractions. Fungal mycelium was collected from the broth using vacuum filtration followed by rinse with sterile-double-distilled water, while still in the funnel and then placed on a lipid-free paper towel for several minutes to remove excess moisture. The lipids were extracted from the dried biomass using chloroform: methanol (2:1 v/v) as per method suggested by Folch *et al*. (2). One gram of fungal mycelium tissue placed into 4.0 ml of a saponification reagent and homogenized with a tissue grinder. The homogenate was then re-extracted with chloroform: methanol (2:1 v/v). The extract of each sample was dried under stream of nitrogen gas, after which the lipid was dissolved in chloroform and methylated. To methylate the liberated fatty acids, 2.0 ml of 54% 6 N HCl in methanol was added to each tube. Sub-samples were then placed in an 80°C water bath for 10 min. followed immediate cooling to room temperature. Prior to FAMEs analyses, the samples were evaporated under nitrogen and resuspended in 50 µl of hexane. The fatty acid methyl esters were analysed using a gas chromatograph (Shimadzu 5050) equipped with mass detector using a 30 m × 0.32 mm, 0.53 µm internal diameters, DB1 fused silica capillary column. The carrier gas was helium at a flow rate of 10 ml/ min. The temperature of the injector was 250°C and that of the detector was 280°C. The oven temperature after sample injection (2 µl) was 1 min. at 115°C, increased to 200°C at 7.5°C/ min. and then finally raised @ 5°C/ min. to 240°C held for 2 min. and then raised at a rate of 3.5°C/ min. to 260°C held at for 2 min. The peaks were identified by reference to authentic standards and verified using mass selective detector.

A total of nine major fatty acids were detected in the two *Fusarium oxysporum* f. sp. isolates. The identified fatty acids were iso-myristic acid (C14:0), pentadecaenoic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0) as saturated and palmitoleic acid (C16:1 n_7), cis-10-heptadecenoic acid (C17:1 n_8), oleic acid (C18:1 n_9), linoleic acid (C18:2 n_6), linolelaidic acid (C18:2 trans), α-linolenic acid (C18:3 n_3), cis-11,14-eicosadienoic (C20:2) and cis-11,14,17-eicostarienoic (C20:3 n_3) as unsaturated ones. Margaric acid (C17:0) was detected though at the same level in the two isolates. The result obtained suggests that the presence or absence of pentadecaenoic acid (C15:0), saturated and palmitoleic acid (16:1 Cis 9 (w 7) and palmitic acid (C16:0) could be used as efficient FAME biochemical markers, for deciphering chemotaxonomic relatedness between two close isolates of *F. oxysporum* f. sp. *gladioli*.

The comparison of means for individual fatty acids of the two isolates was performed and the percentage of fatty acid compositions is given in Table 1. Both the isolates of *F. oxysporum* f. sp. *gladioli* (*Fog*) were quite distinct with respect to the fatty acid profiles and also their percentage compositions. The percentage of an individual fatty acid ranged from less than 1 to over 46% of the total identified fatty acid content. The most common and abundant fatty acids in the two *Fusarium* isolates were palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0) as saturated and palmitoleic acid (C16:1 n_7), cis-10-heptadecenoic acid (C17:1 n_8), oleic acid (C18:1 n_9) and linoleic acid (C18:2 n_6), which accounted for 94.45 and 98.35% of the fatty acid profiles in *F. oxysporum* f. sp. *gladioli* IARI and *F. oxysporum* f. sp. *gladioli* Pantnagar isolates, respectively.

Table 1. Fatty acid composition of *Fusarium oxysporum* f. sp. *gladioli* isolates.

Peak name	IUPAC / Systematic name (Other name)	Per cent	
		IARI isolate	Pantnagar isolate
11:00	Decanoic acid (Undecylic acid)	0.08	0.08
14:00-iso	12-Methyltridecanoic acid (Isomyristic acid)	0.48	0.48
15:0 Anteiso	12-Methyltetradecanoic acid (12-Methylmyristic acid)	0.18	4:19
15:00	Pentadecanoic acid (Pentadecylic acid)	0.39	0.39
16:1 Cis 9 (w 7)	(9Z)-9-Hexadecenoic acid (Palmitoleic acid)	0.46	11:02
16:00	Hexadecanoic acid (Palmitic acid)	14.86	20:38
17:1 Cis 9 (w 8)	(9Z)-9-Heptadecenoic acid	0.39	0.39
17:0 Cyclo	cis-9,10-Methylene-Hexadecanoic acid	0.15	0.15
17:00	Heptadecanoic acid (Margaric acid)	0.46	0.46
18:2 Cis 9,12/18:0a	(9Z)-9-Octadecenoic acid (Oleic acid)	46.24	46.24
Sum in Feature 8		30.43	30.43
18:00	Octadecanoic acid (Stearic acid)	5.04	5.04
Unknown 18.197	--	0.30	0.30
18:1 Cis 11 DMA	(9Z)-1,1-Dimethoxy-9-Octadecene	0.28	0.28
20:00	Icosanoic acid (Arachidic acid)	0.27	0.27
Summed Feature 8		30.43	28.43

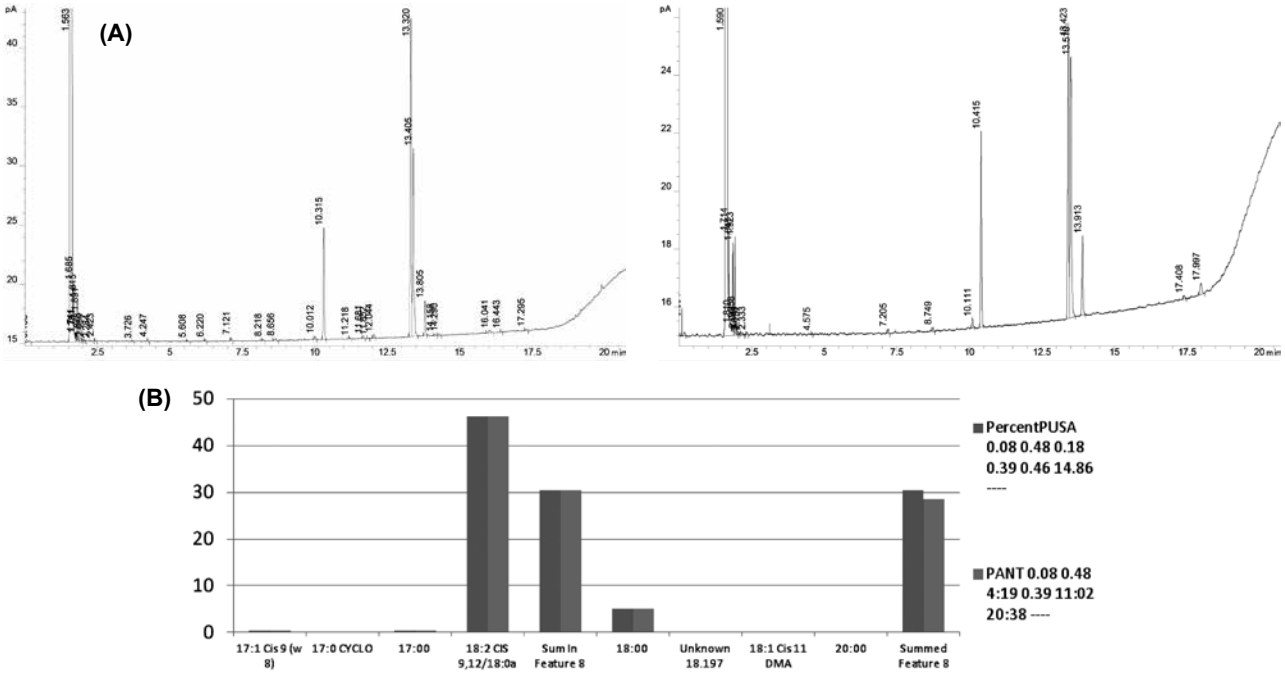

Fig. 1. Comparative composition of fatty acid fractions in IARI and Pantnagar *F. oxysporum* f. sp. *gladioli* isolates. (A) and (B) are the profiles of the *Fog* isolates for comparison of their major fatty acid contents.

The other fatty acids were found as minor fractions in both the isolates. The content of palmitoleic acid was 11.02% in *Fog* Pantnagar isolate, which was much higher compared to *Fog* IARI isolate, *i.e.* 0.46%. The second most predominant fatty acid was palmitic acid (C16:1), which was 14.86% in *Fog* IARI isolate compared to 20:38% in *Fog* Pantnagar isolate. However, in both the isolates, the highest

content was of α-linoleic acid (C18:3 n_3) (46.24%). Significant differences ($p<0.05$) in nine major fatty acid fractions were observed for both the *Fog* isolates (Table 1, Fig. 1).

Chemotaxonomy is traditionally restricted to fatty acids, proteins, carbohydrates, or secondary metabolites, but has sometimes been defined so broadly that it also includes DNA sequences (Schmidt *et al*., 7). The amino acid, fatty acid, and the secondary metabolite profiles of 11 *Fusarium* sp. were determined and assessed to be used as biochemical markers. The results of current study revealed that the secondary metabolite profile was the most effective and informative marker, followed by fatty acid profile, to be applied for taxonomy of the genus *Fusarium*. The genus *Fusarium* and associated teleomorphs in *Gibberella* were examined chemotaxonomically and the species-specific profiles of secondary metabolites were shown to exist (Thrane, 11; Thrane and Hansen, 12; Summerell *et al*., 10). The current study has also confirmed this concept, and the obtained results showed also that numerous secondary metabolites were restricted in distribution and coined to only one species of *Fusarium*. The taxonomy of *Fusarium* species has always been a controversial issue (Nelson *et al*., 6). However, the results obtained from this study strongly suggested that introducing a mixed profile concluded from amino acid, fatty acid, and secondary metabolite profiles would be effective and useful in taxonomy of the genus *Fusarium*. Both the isolates were registered in the Indian Type Culture Collection (ITCC) at the Division of Plant Pathology, ICAR-IARI, New Delhi and also to undertake future research on ornamental crops.

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