Quantitative Comparison of Nuclear DNA Content among Formae Speciales of Fusarium oxysporum and Fusarium solani

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Quantitative Comparison of Nuclear DNA Content among Formae Speciales of *Fusarium oxysporum* and *Fusarium solani*

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SUMMARY

The nuclear DNA content in arbitrary unit was compared by Feulgen-microspectrophotometry to determine genetical relationships among formae speciales of *Fusarium oxysporum* and *F. solani*. Relatively low nuclear DNA content was found in *F. oxysporum* as compared with *F. solani*. It differed significantly between above two species, but not within formae speciales of the same species. These results suggested that quantitative changes in nuclear DNA in *Fusarium* species had occurred in the evolution of the species level, but not the formae speciales level.

INTRODUCTION

Fusarium species are widely distributed throughout the world and are among the fungi most frequently isolated from diseased crops and soils by plant pathologists. The taxonomy of the section *Elegans*, genus *Fusarium*, was extremely simplified by Snyder and Hansen[1], who minimized the significance of the small morphological variations used by Wollenweber[2]. Host specificity and pathogenic pattern are emphasized as the *sole* basis for assigning isolates to taxa termed formae speciales, especially within species *F. oxysporum* (Schl.) emend. Snyder and Hansen which is one of the most important members of the genus *Fusaria* and causes vascular browning and wilting of different host plants. Formae speciales are physiological strains that are indistinguishable from saprophytic strains of the same species, but show different physiological properties in their ability to parasitize specific hosts[1]. Therefore, it would not be possible to identify the isolate as a forma specialis without the inoculation of susceptible varieties of presumptive host species. On the other hand, since the forma exhibits a high degree of host specificity, suitable physiological and biochemical differences, as shown by zymograms, have been used as a criterion to distinguish the taxa in *F. oxysporum* and other species[3,4,5,6,7,8]. Serological methods have also been reported as a basis to differentiate formae speciales or species[9,10,11]. It has been also known that the DNA base composition, expressed as the mean guanine plus cytosine content in moles per cent (% GC) from related organisms, are similar and can be used for taxonomic and phylogenetic purposes in eucaryotic microorganisms including genus *Fusarium*[12,13,14]. The nuclear DNA content also revealed the phylogenetical relationships among related species of higher plants[15,16,17,18,19]. In the earlier paper, the genetic relationship among anastomosis groups of *Rhizoctonia solani* Kühn was discussed on the basis of comparison of nuclear DNA content in their hyphal cells[20].

In this paper, nuclear DNA content was compared among formae speciales of *F. oxysporum* and *F. solani*.

MATERIALS AND METHODS

In this experiment, 13 and 3 formae speciales of *F. oxysporum* and *F. solani* were used, respectively. They were *F. oxysporum* Schl. f. sp. *batatas* (Wr.) Snyd. et Hans., f. sp. *conglutinans* (Wr.) Snyd. et Hans., f. sp. *cucumerinum* Owen, f. sp. *faba* Yu et Fang., f. sp. *fragariae* Winks and Williams, f. sp. *lagenariae* Matuo et Yamamoto, f. sp. *lycopersici* (Sacc.) Snyd. et Hans., f. sp. *melongenae* Matuo et Ishigami, f. sp. *melonis* (Leach et Curr.) Snyd. et Hans., f. sp. *niveum* (Smith) Snyd. et Hans., f. sp. *phaseoli* Kend. et Snyd., f. sp. *raphani* Kend. et Snyd., and f. sp. *spinaciae* (Scherb.) Snyd. et Hans. . As formae speciales of *F. solani* (Mart.) App. et Wr., f. sp. *mori* Sakurai et Matuo, f. sp. *phaseoli* (Burk.) Snyd. et Hans., and f. sp. *pisi* (Jones) Snyd. et Hans. were used. *F. moniliforme* Schld. emend. Snyd. et Hans. was also used to compare nuclear DNA content with the other species.

F. oxysporum f. sp. *conglutinans*, f. sp. *raphani* and f. sp. *spinaciae* were isolated from each diseased host plant, respectively, in Gifu Prefecture. Four formae, f. sp. *lycoperisici, melongenae, melonis* and *cucumerinum* were obtained from Vegetable and Ornamental Crops Research Station, Mie Prefecture, and all the other isolates (SUF) used were obtained from Shinshu University. All isolates were maintained on potato sucrose agar (PSA), and microconidia used as specimens were prepared by growing the monoconidial isolates of each forma for 7- to 10-day on PSA plates and harvesting the conidia in sterilized distilled water. Conidial suspension free from large mycelium fragments by filtration through 3 layer cheese clothes were washed and pelleted by centrifugation. The required spore suspension was prepared by resuspending the pellet in sterilized distilled water.

Microconidia obtained from the respective specimens were smeared on a slide glass, dried at room temperature, and then fixed in Farmer's fluid (acetic acid 1 : 95% ethanol 3) for more than 2 hr. After hydrolysis in 5N HCl at 25℃ for 20 min, the material was stained by buffered Schiff's reagent. Subsequently, the preparations were washed three times in potassium metabisulfite solution, dehydrated in absolute alcohol and mounted in Canada balsam.

Microspectrophotometrical measurements of nuclear DNA content were made at the wavelength of 560 nm with Nikon Vickers M86. More than 30 nuclei for each isolate were measured. As a control, microconidia of *F. oxysporum* f. sp. *spinaciae* were smeared side by side with the respective isolate to adjust the value on each slide.

RESULTS AND DISCUSSION

Mean DNA content per nucleus of formae speciales of *F. oxysporum* and *F. solani* was shown in Table 1 together with the value of *F. moniliforme*. The results in this experiment show that the nuclear DNA content level was significantly different between species *F. oxysporum* and *F. solani*, and it was lower in formae speciales of *F. oxysporum* than in those of *F. solani* and in *F. moniliforme*. However, there were no significant differences at $P=0.05$ in nuclear DNA content within 13 formae speciales of *F. oxysporum* and also within 3 formae speciales of *F. solani* which has the same amount of nuclear DNA as *F. moniliforme*. The variation in nuclear DNA content within a forma speciales was closely correlated with its coefficient of variation, ranging from 8.7 to 27.1% in *F. oxysporum*. Also, no significant differences were found between two different races (J-1 and J-3) which belong to *F. oxysporum* f. sp. *lycopersici* and also between two different isolates (SUF 926 and NF 557) of *F. oxysporum* f. sp. *spinaciae*. Conversely, Madhosingh[5] reported that race 1 and race 2 of *F. oxysporum* f. sp. *spinaciae* redefined by Armstrong and Armstrong[21] are distinguishable on the basis of differences in the patterns of esterase, carbonic anhydrase and acid phosphatase isoenzymes by an electrophoretic comparison of a number of isoenzymes. He also obtained specific immune sera for each race. Although

Table 1. Comparison of mean nuclear DNA content (Arbitrary unit) in microconidia of formae speciales of *Fusarium oxysporum* and *Fusarium solani*.

Isolates tested	DNA content/Nucleus (Arbitrary unit)	(C.V)[1]
Fusarium oxysporum f.sp. *batatus* (SUF 221)	125.0 ± 24.6 a[2]	19.7
F. oxysporum f.sp. *conglutinans*	133.8 ± 13.4 a	9.9
F. oxysporum f.sp. *cucumerinum*	136.4 ± 17.9 a	13.1
F. oxysporum f.sp. *faba* (SUF 888)	132.0 ± 35.8 a	27.1
F. oxysporum f.sp. *fragariae* (SUF 1310)	134.4 ± 17.7 a	13.2
F. oxysporum f.sp. *lagenariae* (SUF 797)	134.1 ± 15.4 a	11.5
F. oxysporum f.sp. *lycopersici* (J-1)	135.5 ± 24.9 a	18.1
F. oxysporum f.sp. *lycopersici* (J-3)	133.5 ± 23.1 a	17.6
F. oxysporum f.sp. *melongenae*	134.3 ± 11.7 a	8.7
F. oxysporum f.sp. *melonis*	128.1 ± 24.5 a	19.1
F. oxysporum f.sp. *niveum* (SUF 366)	133.1 ± 16.3 a	12.2
F. oxysporum f.sp. *phaseoli* (SUF 672)	133.6 ± 18.4 a	13.8
F. oxysporum f.sp. *raphani*	134.9 ± 20.3 a	15.1
F. oxysporum f.sp. *spinaciae* (SUF 926)	123.2 ± 20.2 a	16.4
F. oxysporum f.sp. *spinaciae* (NF 577)	123.6 ± 14.3 a	11.6
Fusarium solani f.sp. *mori* (SUF 232)	182.8 ± 37.3 b	20.4
F. solani f.sp. *phaseoli* (SUF 386)	183.5 ± 55.1 b	30.0
F. solani f.sp. *pisi* (SUF 654)	177.6 ± 65.3 b	36.7
Fusarium moniliforme (SUF 1000)	171.5 ± 17.5 b	10.2

1) Coefficient of variation (%)
2) Means followed by the same letter are not significantly different at $p=0.05$ by Duncan's multiple range test.

Meyer and Renard[8] failed to distinguish *F. oxysporum* f. sp. *melonis* from f. sp. *elaeidis*, it was shown that the esterase zymogram of *F. oxysporum* f. sp. *lycoperisici* was different from that of other formae speciales of the same species[3,6]. Furthermore, Matsuyama and Wakimoto[6] reported that the zymograms of each forma of *F. solani* were almost identical and were distinctly different from those of *F. oxysporum*. As the zymograms of *Fusarium* species were species specific, they concluded that their results agreed with morphological and immunological observations of other workers[9], and confirmed the usefulness and reliability of esterase zymograms for identification and taxonomy of *Fusarium* species.

From the results cited above, it might be possible to distinguish and identify *Fusarium* isolates below the species level by biochemical and serological methods. On the other hand, Storck and Alexopoulos[14] reported that the GC contents analyzed in 8 different species of *Fusarium epishaeria, F. moniliforme, F. moniliforme* var. *minus, F. oxysporum, F. roseum, F. sambucum, F. scirpi* and *F. solani* showed a relatively narrow range from 50 to 53%, irrespective of the great variation exhibited within a species and even within a forma. These results suggest that the qualitative changes of DNA and their recombination might have had a role of minor significance in the evolution of *Fusarium* species. It is also suggested from the results in this experiment that quantitative changes in nuclear DNA had occurred in the evolution of *Fusarium* species, but not in that of the forma specialis of respective species. In this experiment, only nuclear DNA content in microconidia from each forma specialis was measured. More isolates from each forma from diverse geographic sources must be tested and

compared to know in more detail the extent of the variation and the constancy of nuclear DNA content among different stages in the life cycle of the fungi.

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Fusarium oxysporum と *Fusarium solani* の分化型間の核DNA量の比較

内記　隆

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要　約

Fusarium oxysporum と *Fusarium solani* の各々13および3分化型（formae speciales）の小型分生胞子の核DNA量を顕微濃度計を用いて測定，比較した。その結果 *F. oxysporum* の核DNA量は *F. solani* のそれより低く，両種間に核当りDNAの量的変化が認められた。一方同一種内の分化型の間では核当りDNAに量的差異は認められず，核DNA量からみると分化型は極めて近縁であった。