Asian Journal of Agricultural Sciences 3(3): 156-161, 2011
ISSN: 2041-3890

Received: January 05, 2011 Accepted: January 31, 2011 Published: May 25, 2011

Pea Footrot Disease Depends on the Combination of Pathogenicity Genes in *Nectria haematococca*

[1]Ebimieowei Etebu and [2]A. Mark Osborn
[1]Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
[2]Department of Biological Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX United Kingdom

Abstract: Footrot disease due to *Nectria haematococca* is an economically important disease of peas all over the world where peas are grown. The combined effect of pathogenicity genes on disease severity has not been adequately addressed. Hence in this research, molecular PCR-based assays have been developed and/or used to detect all six (*PDA*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5*) pea pathogenicity genes in fifteen fungal isolates previously isolated from fields with footrot disease. The pathogenicity of these isolates on pea was also assessed. Results showed that all six pathogenicity genes (*PDA*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5*) are required for high virulence (DI ≥ 3.75). Isolates that possessed only the *PEP1* or a combination of the *PEP1* and *PEP4* genes generally caused a relatively low degree of disease (DI≤2.75). Similarly, reference isolate 156-30-6 which possesses only the *PDA* gene was non pathogenic on peas (DI = 1.50±0.87). However, without exception, isolates that possessed the *PDA* gene alongside other gene(s), especially *PEP2*, *PEP3* and *PEP5* genes caused high degrees of disease (DI≥3.50). The *PEP2*, *PEP3* and *PEP5* genes were observed only in isolates with high pathogenicity (DI≥3.75).

Key words: Footrot disease, *Fusarium solani*, *Nectria haematococca*, pathogenicity genes, pea, *Pisum sativum*

INTRODUCTION

Foot rot is an acknowledged economically important disease of a number of major commercial crops in the UK, including peas. The main causative agents of foot rot in peas are *Nectria haematococca* (anamorph *Fusarium solani* f. sp. *pisi*) and *Phoma medicaginis* var. *pinodella*, and to a lesser degree *Mycosphaerella pinodes* (telomorph *Ascochtya pinodes*), and some species of *Pythium* (Hagedorn, 1991).

Footrot disease of peas due to *N. haematococca* MPIV has been linked to the presence of six pea pathogenicity (*PEP*) genes (*PDA1*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5*) (Temporini and VanEtten, 2002). These *PEP* genes are clustered together on a supernumary or conditionally dispensable (CD) 1.6 Mb chromosome (Han *et al*., 2001, Temporini *et al*., 2002; VanEtten *et al*., 1994). Molecular techniques have been successfully used to identify, and demonstrate the presence of these genes in DNA extracted from pure strains of *N. haematococca*, virulent on peas 9 Temporini and VanEtten, 2002). These techniques have been recently used to identify some of the pea pathogenicity genes (*PDA*, *PEP1*, *PEP3* and *PEP5*) in virulent strains of *N. haematococca* in the UK (Etebu and Osborn, 2009). Although the sequences of these genes in pathogenic forms of *F solani* f. sp. *pisi* are closely related, irrespective of the country where they occur (Temporini and VanEtten, 2002), the fungal isolates possessing the genes cause varying severities of footrot disease on peas. The differing degrees of pathogenicity appeared to have been influenced by variation in the presence or absence, and/or the combination of pathogenicity genes detected in each isolate by Etebu and Osborn (2009). The combined effects of different combinations of these genes on pea footrot disease have not been adequately addressed because a recent study (Etebu and Osborn, 2009) which attempted to do so considered only four of the known six genes. From the ongoing, it is imperative to assess the presence/absence of all six pea pathogenicity genes in fungal isolates to properly determine the influence of the genes on its pathogenicity on peas.

Hence in the research, the pathogenicity of fifteen isolates previously studied by Etebu and Osborn (2009) were assessed. Also, molecular PCR-based assays have been developed and/or used to screen all fifteen and two

Corresponding Author: Ebimieowei Etebu, Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

Table 1: Oligonucleotide primers used to amplify *PDA*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5* gene sequences

Gene	Primer pair	Primer sequence (5'-3')[a]	Amplicon size (bp)	Reference
PDA	PDAF2	TGC GTC TCT CTT CAC TCC TAC CG	301	Etebu and Osborn (2009)
	PDAR2	CGA AGA GTG TGC AGA GTA CGT GG		
PEP1	PEP1F1	CAA GTT TCA GCT CAT CCA CAG GC	301	Etebu and Osborn (2009)
	PEP1R1	TTT CTC CTT GAC GTG CCA AAT C		
PEP2	PEP2F1	AGA TAT CAA CGT CGA TGG AAC GC	301	This study
	PEP2R1	CAG TAC CTT GAT ACC CAT CGC G		
PEP3	PEP3F1	CAG AAC CTC AGT CAT GCT TCA ACA C	151	Etebu and Osborn (2009)
	PEP3R1	GCT TGA GAA GCC ATT TTG GGT CT		
PEP4	PEP4F1	AAC GCA CAT CAG AGG ATT CTT CG	301	This study
	PEP4R1	AAT ACT CCT CTG TGA CGC GTT TTG		
PEP5	PEP5F2	GAT GGC TGG AAT GGG GCT CT	298	Etebu and Osborn (2009)
	PEP5R2	GGC GGT GTA GAC AGG AAG AG		

[a]: Primer sequences (Top = forward primer;Bottom = reverse primer) were designed to target consensus sequences in pathogenicity genes from *N. haematococca* (accession numbers AF294788 and AF315315)

reference isolates to determine the presence or absence of any or all of the six (*PDA*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5*) pea pathogenicity genes. The combined effects of these genes in fungal isolates on the severity of footrot disease in peas are herein discussed.

MATERIALS AND METHODS

Growth assays to ascertain fungal pathogenicity: Fifteen fungal isolates whose pea pathogenic status had been previously determined by Etebu and Osborn (2009) were used to artificially infect viable Pea seeds to ascertain pathogenic status of the fungi. Fungal pathogenicity assays were performed as described by Etebu and Osborn (2009) with slight modifications. Greenshaft pea seeds purchased from Monks Farm, Kelvedon Colchester, Essex CO5 9PG, United Kingdom, and generously provided by Mr Daniel Kinsman, Department of Animal and Plant Sciences, University of Sheffield were first surfaced sterilised in 10% economy bleach for 5mins and rinsed repeatedly with sterile tap water (adapted from Etebu *et al*., 2003). Seeds were thereafter sandwiched between sheets of sterilized filter papers moistened in sterile water, and incubated in the dark at 23ºC for 3 days. At the end of 3 days viable seeds were ascertained by the visible protrusion of their radicules. Viable pea seeds were placed on the surface of 0.8% water agar in boiling tubes. 1 mL conidial suspensions (10^4 conidia in 0.5% Tween) of each fungal strain were separately inoculated onto the peas (n = 4 per fungal strain) and then incubated in the dark at 22ºC for seven days. Viable peas were alternatively infected with either *N. haematococca* (anamorph *F. solani* f. sp. *pisi*) strains 77-13-7 or 156-30-6. Strain 77-13-7 is highly pathogenic on peas whilst strain 156-30-6 is non-pathogenic (Miao *et al*., 1991a, b). Additionally, peas were also grown on culture medium inoculated with 0.5% Tween without fungal conidia as a control. Root disease was assessed after seven days using a disease index (DI) scale of 0-5 (Etebu and Osborn, 2009; Oyarzun *et al*., 1997; VanEtten and Kistler, 1988).

Extraction of total nucleic acids from fungal isolates; Total nucleic acids were extracted from mycelia of sporulating isolates using a modification of the method of Griffiths *et al*. (2000). Approximately 0.6 g of mycelia was frozen in liquid nitrogen prior to lysis by bead beating. Fungi were mixed with 0.5 mL of extraction buffer (5% cetyltrimethylammonium bromide (CTAB), 0.35 M NaCl, 120 mM potassium phosphate pH 8.0) and 0.5 ml of phenol-chloroform-isoamyl alcohol (25:24:1; pH 8.0) and 0.5 g of glass beads and 1 grinding bead (~0.5 cm diameter) and lysed by bead beating for 1 min at 1,425 rpm using a 8000M mixer/mill (Spex Certiprep, Middlesex, UK), prior to centrifugation at 7000 g for 5 min. The upper aqueous phase containing nucleic acids was then mixed with an equal volume of chloroform-isoamyl alcohol (24:1) followed by centrifugation (7,000 g) for 5 min, and then nucleic acids in the aqueous phase were precipitated with two volumes of 30% (wt/vol) polyethylene glycol 6000 in 1.6 M NaCl for 2 h at room temperature. Nucleic acids were pelleted by centrifugation at 11,000 g for 10 min, washed with 400 μL of ice cold 70% ethanol, air dried and resuspended in 25 μL of sterile nuclease-free water.

Polymerase chain reaction (PCR) amplification of pea pathogenicity genes: Partial sequences of Pea pathogenicity genes (*PDA*, *PEP1*, *PEP3* and *PEP5*) were amplified with primers previously developed by Etebu and Osborn (2009). To target *PEP2* and *PEP4* genes, primer pairs were designed using the ABI Primer express software (Applied Biosystems, California, USA) (Table 1). PCR amplifications were performed in reactions containing 1 μL of template DNA, 5 μL of 10x PCR buffer (Bioline), 1.25 mM $MgCl_2$, 200 μM of

Table 2: Pea pathogenicity and carriage of pathogenicity genes by fungal isolates

S.No.	IsolateCode	Conidialform[a]	Mean DI[x](n = 4)	Pathogenicity genes detected by PCR[y]					
				PDA	*PEP1*	*PEP2*	*PEP3*	*PEP4*	*PEP5*
1	A04	+	1.50 ± 0.50	-	+	-	-	-	-
2	A13	-	2.00 ± 0.71	-	+	-	-	+	-
3	B22	+	3.50 ± 0.50	+	+	-	-	+	-
4	B27	+	3.50 ± 0.50	+	-	-	-	+	-
5	B29	+	4.25 ± 0.48	+	+	+	+	+	+
6	B30	+	3.75 ± 0.48	+	+	+	+	+	+
7	C31	+	4.50 ± 0.50	+	+	+	+	+	+
8	C37	+	1.50 ± 0.50	-	+	-	-	+	-
9	D49	+	3.25 ± 0.62	-	+	-	-	-	-
10	D51	+	2.50 ± 0.96	-	+	-	-	+	-
11	D53	+	2.50 ± 0.65	-	+	-	-	+	-
12	D54	-	0.75 ± 0.48	-	-	-	-	-	-
13	D58	+	2.75 ± 0.85	-	+	-	-	+	-
14	D60	-	1.50 ± 0.87	-	-	-	-	-	-
15	E73	-	0.25 ± 0.25	-	-	-	-	-	-
16	77-13-7[z]	+	4.50 ± 0.50	+	+	+	+	+	+
17	156-30-6[z]	+	1.50 ± 0.87	+	-	-	-	-	-
18	Control[z]	N/A	0.25 ± 0.25	N/A	N/A	N/A	N/A	N/A	N/A

[a] + or – refers to typical or atypical *Fusarium* conidial morphologies, respectively. Typical fusarial conidia were “canoe-shaped”, divided by several cross-walls, and had a distinct "foot cell" at the lower end

[x] Mean DI (disease index), assessed by extent of root discolouration of peas grown on 0.8% tap water inoculated with fungal strains (n = 4); 0 = no discolouration; 1 = up to 20%; 2 = 21-40%; 3 = 41-60%; 4 = 61-80%; 5 = above 80% discolouration

[y] + or - = refers to genes detected or not detected

[z] Controls: peas were inoculated with reference strains of *Nectria haematococca* (Miao *et al*., 1991a, b) or uninoculated

dNTPs, 20 pmol of each primer, 1U of Taq polymerase (Bioline) and made up to a final volume of 50 µL with sterile nuclease free water. PCR cycling conditions for *PDA*, *PEP2*, *PEP3* and *PEP4* gene sequences were performed as described by Etebu and Osborn (2009). Briefly, the template DNA contained in the PCR reaction mix was denatured at 95ºC for 5 min followed by 35 cycles of 94ºC (1 min), 57ºC (1 min), and 72ºC (1 min) and a final extension at 72ºC for 10 min. PCR cycling conditions for amplification of *PEP1* and *PEP5* genes were 95ºC for 5 min, 5 cycles of 94ºC (45 sec), 55ºC (1 min) and 72ºC (30 sec), followed by 30 cycles of 94ºC (30 sec), 55ºC (45 sec) and 72ºC (30 sec) and a final extension at 72ºC for 10 min. PCR products (8-10 µL) were visualized under UV light at 302 nm following agarose gel electrophoresis.

RESULTS

The virulence of the 15 fungal strains previously isolated from agricultural fields with footrot history was assessed, and six sets of primers (*PDA*F2/*PDA*R2, *PEP1*F1/*PEP1*R1, *PEP2*F1/*PEP2*R1, *PEP3*F1/*PEP3*R1, *PEP4*F1/*PEP4*R1 and *PEP5*F2/*PEP5*R2) targeting genes (*PDA*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5*, respectively) (Table 1) responsible for footrot disease on peas Pea footrot disease were used in PCR-based assays to screen for the presence or absence of the pea pathogenicity genes in the isolates.

Five out of the 15 test isolates, and one of the reference isolates (156-30-6) were non pathogenic (DI ≤ 1.50) (Table 2). Amplicon sizes of PCR product were 301bp (*PDA*), 301bp (*PEP1*), 301 (*PEP2*) 151bp (*PEP3*), 301bp (*PEP4*) and 298bp (*PEP5*) (Fig. 1). Pathogenicity genes were detected by PCR in 12 of the 15 fungal isolates (Table 2, Fig. 1). The fungal isolates were observed to cause varying severities of footrot disease to peas, depending on the combination of pathogenicity genes detected in each isolate (Table 2).

The *PDA* gene was amplified by PCR from five of the fifteen fungi, and the reference isolates; Cumulatively, they caused higher levels of disease severity on peas than those fungi that lacked a *PDA* gene, mean disease indices were 3.64 and 1.75, respectively (Table 2). Whilst all genes were detected with one of the reference isolates (77-13-7), only the *PDA* gene was detected with isolate (156-30-6). This latter reference isolate was observed to lack all other pea pathogenicity genes. Whilst the disease index for isolate 77-13-7 was 4.50±0.5, the disease index for 15-30-6 was 1.50±0.87. The *PEP1* gene was detected by PCR in 11 of the 15 test fungi, and one of the references isolates (77-13-7) and these isolates had a mean disease index of 3.04. The *PEP4* gene was observed in 10 out of the fifteen test fungal isolates with a mean disease index of 3.20±0.31. Majority of isolates that possess the *PEP1* gene also had the *PEP4* gene, and generally caused a low degree of disease (Table 2). The *PEP2*, *PEP3* and *PEP5* genes were all detected in only three of the isolates (B29, B30 and C31), from which *PDA* and *PEP1* and *PEP4* genes were also detected. These isolates caused highest degrees of disease (mean DI = 4.25±0.18) (Table 2).

Whilst isolates that had none of the pea pathogenicity gene were observed to be non-pathogenic

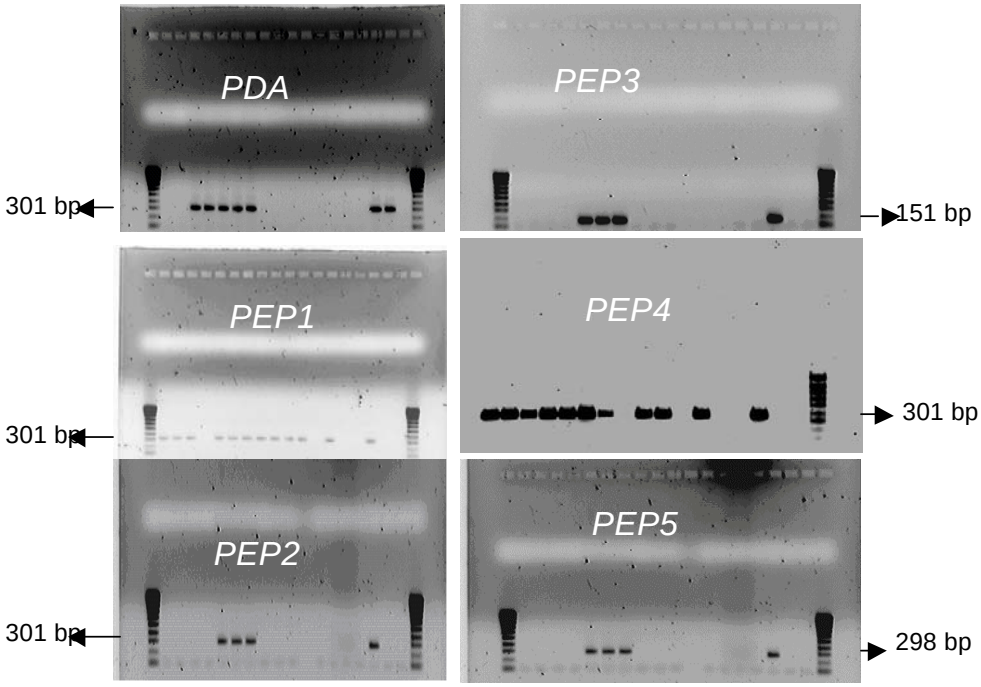

Fig. 1: Agarose gel electrophoresis of pathogenicity genes (*PDA*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5*) amplified by PCR from DNA extracted from fungal/reference isolates Lanes 1) Hyperladder IV 2) A04, 3) A13, 4) B22, 5) B27, 6) B29, 7) B30, 8) C31, 9) C37, 10) D49, 11) D51, 12) D53, 13) D54, 14) D58, 15) D60, 16) E73, 17) 77-13-7, 18) 156-30-6, 19) sdH_20, 20) Hyperladder IV
*: *PEP4* Lane 1 begins with fungal isolate A04 through lane 19 with Hyperladder IV

(mean DI = 0.83), isolates that possessed one or more PEP gene(s) cumulatively caused disease (mean DI = 3.77). Fungal isolates that possessed all six pea pathogenicity genes caused the highest disease severity on peas, mean disease index of 4.50±0.18 (Table 2).

DISCUSSION

The virulence of the 15 fungal strains previously isolated from agricultural fields with footrot history was assessed. Two new sets of primers were designed in this study and used alongside four sets of primers previously designed by Etebu and Osborn (2009) to screen fifteen fungal isolates for the presence/absence of all known six pea pathogenicity genes (*PDA*, *PEP1*, *PEP2*, *PEP3*, *PEP4* and *PEP5*) (Table 1). Pea footrot symptoms were characterized by a reddish brown to black discolouration of the root system around the area of cotyledon attachment (data not shown), similar to those described earlier by other researchers (Kraft, 2001; Kraft and Boge, 2001)

Pathogenicity genes were detected by PCR in 12 of the 15 fungal isolates (Table 2). An earlier work had shown that the amplified pathogenicity gene sequences from these isolates were highly (≥88%) similar to those present in *N. haematococca* (accession numbers L20976, X73145, AF294788, AF315315, EU436558-EU436574) (Etebu and Osborn, 2009). It had been shown that only isolates with conidial morphologies typical of *Fusarium* spp. possessed pea pathogenicity genes, the only exception being isolate A13 (Etebu and Osborn, 2009). Results from this work revealed that in addition to the *PEP1* gene previously observed by Etebu and Osborn (2009), this isolate also possessed the *PEP4* gene (Table 2). With the exception of isolate D49, isolates that possessed only the *PEP1* or a combination of the *PEP1* and *PEP4* genes only caused a relatively low degree of disease (disease index ≤ 2.75). This suggests that the *PEP1* and *PEP4* genes contribute very minimally to pathogenicity. The *PEP4* gene, unlike most of the *PEP* cluster of pea pathogenicity genes, lacks the ability to independently confer pathogenic properties to non-pathogenic isolates of *N. haematococca* that lack the conditional dispensable chromosome (Han *et al*., 2001). Also, Temporini and VanEtten (2002) showed that the *PEP4* gene has little significance to pea footrot disease. Although the *PEP1* gene has been shown to independently confer pea pathogenicity in non pathogenic strains (Ciuffetti and VanEtten, 1996; Han *et al*., 2001), isolates required the *PDA* gene to cause and appreciable disease severity (DI ≥ 3.50) (Table 2).

The *PDA* gene encodes a cytochrome P450 monooxygenase that demethylates the pea phytoalexin

pisatin hence converting the latter to a less toxic form (Matthews and VanEtten, 1983). Three phenotypic groups of *F. solani* f. sp. *pisi* have been identified with respect to possession of *PDA* genes (Matthews and VanEtten, 1983) namely PDA^{-}, PDA^{L} and PDA^{H}. The first group lack the ability to detoxify pisatin (PDA^{-}). The second group produce low levels of pisatin demethylase after long exposure to pisatin (PDA^{L}) while the third group rapidly produce moderate to high levels of pisatin demethylase on exposure to pisatin (PDA^{H}) (Kistler and VanEtten 1984; Mackintosh *et al*., 1989; Tegtmeier and VanEtten, 1982; VanEtten *et al*., 1994.). Recent studies had shown that reference isolate 156-30-6 possessed a structural gene previously associated with a PDA^{L} (Etebu and Osborn, 2009).

Although the importance of the *PDA* gene in pea pathogenicity has been amply established and known, findings from this work showed that the *PDA* gene requires at least one or more of the rest of *PEP* for high virulence (Table 2). This suggests that although the role of the *PDA* gene in pea pathogenicity is recognised, it does not by itself alone cause severe pea footrot disease. One of the reference isolates 156-30-6 which was observed to possess only the *PDA* gene, caused a very low disease on peas (DI = 1.50±0.87). Miao *et al*. (1991a, b) had shown in an earlier work that this isolate was non pathogenic. However, its possession of the *PDA* gene indicates that the detection of the *PDA* may not suffice to predict pathogenicity of an isolate or an agricultural field with high disease potential. However, without exception, isolates that possessed the *PDA* gene alongside other gene(s) were observed to cause an appreciably high degree of disease (DI≥3.50). This observation held sway, irrespective of the allelic form of the *PDA* gene. Etebu and Osborn (2009) showed that the fungal isolates (B29, B30, C31 and 77-13-7) that caused maximum pea footrot disease possessed the *PDA*, *PEP1*, *PEP3* and *PEP5* genes. Findings from this work, however, showed that these isolates, in addition to the aforementioned genes, also possessed the *PEP2* and *PEP4* genes. Hence, this work revealed the presence of all six pea pathogenicity genes in these isolates, confirming the hypotheses that suggest that highest levels of pathogenicity on peas are caused by isolates containing the complete suite of pathogenicity genes.

Although the findings of this and other previous works have shown that highest levels of pea pathogenicity are caused by isolates that possess all known six pea pathogenicity genes, there is still need to determine the gene that would be most suitable to target in screening for isolates or agricultural fields with high pea footrot disease potential. The *PEP2*, *PEP3* and *PEP5* genes were observed to be present exclusively in isolates B29 B30, C31 and 77-13-7 which also caused the highest levels of disease (DI≥3.75) (Table 2). Of these, the *PEP2* and *PEP5* genes are able to independently confer pathogenic properties to non-pathogenic isolates of *N. haematococca* that lack the conditional dispensable chromosome whereon pea pathogenicity genes reside (Han *et al*., 2001). In contrast, the *PEP3* gene lacks the ability to confer pathogenic properties to non pathogenic isolates of *N. haematococca*.

The *PEP3* gene, however, is the only gene in the pea pathogenicity (*PEP*) gene cluster whose homologue(s) are absent in members of the *Fusarium oxysporum* species complex. All of the other *PEP* genes have their homologues present (Temporini and VanEtten, 2004). One may therefore inadvertently detect/quantify *F. oxysporum* if a *PEP* gene other than the *PEP3* gene is targeted in a molecular assay. Additionally, the *PEP2* and *PEP5* genes, unlike the *PEP3* gene, have been observed in isolates that were not highly virulent (Han *et al*., 2001; Temporini and VanEtten, 2002). Although, the role of the *PEP3* gene is yet unknown, and whilst it lacks the ability to independently confer pathogenic properties to non pathogenic isolates of *N. haematococca*, it is the only *PEP* gene that is exclusive to highly virulent strains of *N. haematococca* (Han *et al*., 2001; Temporini and VanEtten, 2002).

The findings of this work, coupled with results of previous works show that the *PEP3* gene would be the most suitable gene to target in screening for isolates or agricultural fields with high pea footrot disease potential. The extension of the molecular assay targeted at the *PEP3* gene to generate quantitative data from soil-DNA would be desirable to quantitatively predict pea footrot infections in agricultural soils prior to cultivation.

ACKNOWLEDGMENT

The authors would like to thank Professor Hans D. VanEtten, University of Arizona, U.S.A for generously providing reference strains of *Nectria haematococca*

REFERENCES

Ciuffetti, L.M. and H.D. VanEtten, 1996. Virulence of a pisatin demethylase deficient *Nectria haematococca* MPVI isolate is increased by transformation with a pisatin demethylase gene. Mol. Plant Microbial. In., 9: 787-792.

Etebu, E. and A.M. Osborn, 2009. Molecular assays reveal the presence and diversity of genes encoding pea footrot pathogenicity determinants in *Nectria haematococca* and in agricultural soils. J. Appl. Microbiol., 106: 1629-1639.

Etebu, E., C. Pasberg-gauhl, F. Gauhl and L.A. Daniel-Kalio, 2003. Preliminary studies on *in vitro* stimulation of sexual mating among isolates of *mycosphaerella fijiensis* Morelet, causal agent of black sigatoka disease in bananas and plantains. Phytoparasitica, 31: 69-75.

Griffiths, R.I., A.S. Whiteley, A.G. O'Donnell and M.J. Bailey, 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA and rRNA-based microbial community composition. Appl. Environ. Microbiol., 66: 5488-5491.

Hagedorn, D.J., 1991. Handbook of Pea Diseases, Report No. A1167. Madison, WI: University of Wisconsin-Extension, Madison, U.S.A.

Han, Y., X. Liu, U. Benny, H.C. Kistler and H.D. VanEtten, 2001. Genes determining pathogenicity to pea are clustered on a supernumerary chromosome in the fungal plant pathogen *Nectria haematococca*. Plant J., 25: 305-314.

Kistler, H.C. and H.D. VanEtten, 1984. Regulation of pisatin demethylation in *Nectria haematococca* and its influence on pisatin tolerance and virulence. J. Gen. Microbiol., 130: 2605-2613.

Kraft, J.M., 2001. Fusarium Root Rot. In: Kraft, J.M. and F.L. Pfleger (Eds.), Compendium of Pea Diseases. St. Paul, MN, USA the Am. Phytopathol. Soc., pp: 13-14.

Kraft, J.M. and W. Boge, 2001. Root characteristics in pea in relation to compaction and fusarium root rot. Plant Dis., 85: 936-940.

Mackintosh, S.F., D.E. Matthews and H.D. VanEtten, 1989. Two additional genes for pisatin demethylation and their relationship to the pathogenicity of *Nectria haematococca* on pea. Mol. Plant Microb. In., 2: 354-362.

Matthews, D.E. and H.D. VanEtten, 1983. Detoxification of the phytoalexin pisatin by a fungal cytochrome-P-450. Arch. Biochem. Biophys., 224: 494-505.

Miao, V.P.W., S.F. Covert and H.D. VanEtten, 1991a. A fungal gene for antibiotic resistance on a dispensable ("B") chromosome. Science, 254: 1773-1776.

Miao, V.P.W., D.E. Matthews and H.D. VanEtten, 1991b. Identification and chromosomal locations of a family of cytochrome-P-450 genes for pisatin detoxification in the fungus *Nectria haematococca*. Mol. Gen. Genet., 226: 214-223.

Oyarzun, P.J., G. Dijst, F.C. Zoon and P.W.Th. Maas, 1997. Comparison of soil receptivity to *Thielaviopsis basicola*, *Aphanomyces euteiches*, and *Fusarium solani* f. sp. *pisi* causing root rot in pea. Phytopathology, 87: 534-541.

Tegtmeier, K.J. and H.D. VanEtten, 1982. The role of pisatin tolerance and degradation in the virulence of *Nectria haematococca* on peas - a genetic analysis. Phytopathology, 72: 608-612.

Temporini, E.D. and H.D. VanEtten, 2002. Distribution of the pea pathogenicity (*PEP*) genes in the fungus *Nectria haematococca* mating population VI. Curr. Genet., 41: 107-114.

Temporini, E.D. and H.D. VanEtten, 2004. An analysis of the phylogenetic distribution of the pea pathogenicity genes of *Nectria haematococca* MPVI supports the hypothesis of their horizontal transfer and uncovers a potentially new pathogen of garden pea: *Neocosmospora boniensis*. Curr. Genet., 46: 29-36.

VanEtten, H.D. and H. Kistler, 1988. *Nectria haematococca*, mating population I and VI. Adv. Plant Pathol., 6: 189-206.

VanEtten, H.D., D. Funnel-Baerg, C. Wasmann and K. McCluskey, 1994. Location of pathogenicity genes on dispensable chromosomes in *Nectria haematococca* MPVI. *Antonnie Leuwen.* Int. J. Microbiol., 65: 263-267.