Random Amplified Polymorphic DNA Markers in Sweetpotato Infected with *Fusarium lateritium*

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Abstract. DNA isolated from *Fusarium lateritium* Nees: Fr.-infected 'Jewel' sweetpotato [*Ipomoea batatas* (L.) Lam.] plants was compared to *F. lateritium*-free 'Jewel' plants for differences in random amplified polymorphic DNA (RAPD) marker products. Differences in RAPD marker products were detected. Amplified DNA isolations from *F. lateritium*-infected 'Jewel' plants generated additional, unique DNA fragments not found in amplified DNA isolations of *F. lateritium*-free 'Jewel' plants. These unique amplified DNA fragments were consistent with those obtained from amplified DNA isolations of the *F. lateritium* isolate, 91-27-2, used for inoculation. We found that *F. lateritium* DNA successfully competes with sweetpotato DNA in the polymerase chain reaction for priming sites in a 3 : 1 ratio of sweetpotato DNA to *F. lateritium* DNA. Our results indicate the importance of avoiding plant material infested with pathogens to avoid spurious marker bands.

Random Amplified Polymorphic DNA (RAPD) markers are routinely used to construct linkage maps (Yu et al., 1993) and assess genetic diversity and systematic relationships (Jarret and Austin, 1994; Wachira et al., 1995) in a wide array of species. Additionally, DNA-based markers are widely suggested as means of unambiguously identifying cultivars for proprietary descriptions (Jondle, 1992; Rongwen et al., 1995) and, recently, determining the purity of hybrid seed (Paran et al., 1995). The RAPD technique has supplanted RFLP and isozyme techniques in many of these applications because of its lower cost, technical simplicity, high level of polymorphisms (Weeden et al. 1992), and low DNA template requirements.

Most researchers typically assume that any foreign microorganism's DNA present on in situ plants competes poorly for priming sites (Williams et al., 1993) or will be quantitatively low and will not appreciably amplify

and appear as artifacts. In this report we show that RAPD markers unique to the ubiquitous exopathogen *Fusarium lateritium* amplify

along with those of sweetpotato infected with this disease, commonly known as chlorotic leaf distortion (CLD) (Clark et al., 1990).

Materials and Methods

Indexed (Moyer et al., 1989), meristem-tip cultured 'Jewel' explants free of *F. lateritium* and sweetpotato feathery mottle virus were grown in a screenhouse. A portion of these explants were inoculated with a suspension of 1.0×10^6 macroconidia and microconidia of *F. lateritium* isolate 91-27-2. Mild chlorotic leaf distortion (CLD) symptoms, i.e., white fungal hyphae along leaf margins and mild chlorosis on the youngest terminal leaf, were observed beginning 5–7 weeks after inoculation. Three to four terminal, opened leaves were then removed from each infected plant and from isolated plants free of *F. lateritium*. Terminal leaves also were harvested from field-grown 'Jewel' clones naturally infected with *F. lateritium*; symptoms on these were more severe than on greenhouse material, with chlorosis extending to three to four terminal opened leaves.

Total DNA from ≈ 2 g of freeze-dried leaf tissue from each sample plant was isolated based on the method of Saghai-Marroof et al. (1984) as modified by Jarret and Austin (1994). Conditions for DNA amplification using polymerase chain reaction (PCR) and electrophoresis have been reported by Villordon and La Bonte (1995). All RAPD primers that produced amplified DNA fragments were retested for reproducibility. Only intense staining bands were scored.

Fusarium lateritium isolate 91-27-2 was

Table 1. Effect of *Fusarium lateritium* infection on the occurrence of RAPD markers in 'Jewel' sweetpotato.

DNA marker ^y	Amplified DNA product ^z			<i>Fusarium lateritium</i> ^w
	CLD+ in situ Jewel ^x	CLD- Jewel	CLD+ Jewel	
F12-2127	+	–	+	+
F12-1878	+	–	+	+
F12-1085	–	–	–	+
F12-898	+	+	+	–
F12-788	+	+	+	–
F12-640	+	+	+	–
F1-1549	+	+	+	–
F1-1468	–	–	–	+
F1-1391	+	+	+	–
F1-1186	+	+	+	–
F1-1066	+	–	+	+
F1-957	–	–	–	+
F1-857	+	–	+	+
F1-643	+	+	+	–
F1-405	+	+	+	–
A16-2896	+	–	+	+
A16-1925	–	–	–	+
A16-1634	+	–	+	+
A16-1468	–	–	–	+
A16-1392	+	+	+	–
A16-1125	+	+	+	–
A16-436	+	+	+	–

^zPresence of amplified DNA product; – absence of amplified DNA product.

^yProvisional marker designation represents Operon primer kit designation plus the estimated fragment size in bp.

^xCLD+ chlorotic leaf distortion affected, CLD- chlorotic leaf distortion free; in situ Jewel represents a 'Jewel' clone naturally infected under field conditions, CLD+ and CLD- Jewel represent greenhouse grown mericlones.

^wIsolate 91-27-2.

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cultured at 28 °C in potato dextrose broth (PDB) on a shaker set at 200 rpm. for 1 week before vacuum filtration on Whatman #1 filter paper. Total DNA was extracted, as before, from mycelium and bud cells weighing ≈0.5 g. Reaction volumes for *F. lateritium* DNA were adjusted to 40 ng/25 µl. Amplification and electrophoresis conditions were the same as for plant DNA.

Results and Discussion

Three 10-mer oligonucleotide primers, OPA16, OPF1, and OPF12 (Operon Technologies, Alameda, Calif.), respectively, amplified 3, 5, and 3 DNA fragments in the PCR mixture of DNA isolated from *F. lateritium*-free 'Jewel' plants. These fragments were consistent with those observed in previous studies with 'Jewel' (Villordon and La Bonte, 1995). Utilization of DNA isolated from *F. lateritium*-infected 'Jewel' plants in the PCR mixtures resulted in the amplification of unique DNA fragments not present when the template DNA was isolated from *F. lateritium*-free 'Jewel' plants (Table 1). These amplified DNA fragments were identical to those generated from the *F. lateritium* template DNA. Three additional primers, out of 35 originally screened, amplified faint DNA fragments that fit the same general banding pattern (data not shown). DNA fragments amplified by PCR from field-grown 'Jewel' infected with naturally-occurring *F. lateritium* were identical to those from inoculated 'Jewel' (Table 1). This result demonstrates that our results were not merely artifacts of greenhouse conditions.

Preliminary studies showed that *F. lateritium* DNA template concentrations as low as 10 ng/25 µl of reaction volume were

sufficient to generate amplification products after 40 cycles of PCR. Our results indicate that *F. lateritium* DNA reaches concentrations that approach or exceed this amount in infected sweetpotato DNA isolations adjusted to 40 ng of total DNA/25 µl of reaction volume; however, amplifications at lower DNA template concentrations are possible if many high- and middle-repetitive sequences are present or perfect inverted repeats exist in the *F. lateritium* genome. We also found that *F. lateritium* competes successfully for priming sites. A 3 : 1 ratio of sweetpotato DNA to *F. lateritium* DNA in PCR mixtures produced amplified DNA fragments specific to both species.

We have shown that PCR amplification using total DNA extracts from *F. lateritium*-infected sweetpotato plants produce DNA fragments specific to the host and pathogen. Our results imply that other plant species may harbor pathogens that may produce DNA fragments that might confound analyses and interpretation.

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