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CONVERSION OF RAPD MARKERS INTO SCAR MARKERS FOR A STRAIGHTFORWARD DIAGNOSIS OF POTATO INFECTION WITH *PHYTOPHTHORA INFESTANS*

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Abstract. In our researches we tried to convert the *P.infestans* RAPD markers into SCAR markers for a more operative diagnosis of potato late blight in the infected material. For this goal, several monomorphic and polymorphic RAPD sequences were recuperated from the agarose gel, and cloned into *E.coli* bacteria. The cloned monomorphic sequences were sequenced, establishing the nucleotide sequence. Relying on this several pair of primers was designed. One pair from them, with the following nucleotide sequences AGC CCA CAC TGA AAT TAC GC/GGC TAC CGG ATC AGT ACC AA), gave amplification product only with *P.infestans* DNA. Being specific, this pair pf primer could be used for a straightforward potato late blight diagnosis from the infected material, and our objective concerning the conversion of *P.infestans* RAPD markers into SCAR markers could be considered accomplished.

INTRODUCTION

Late blight is the most important disease of potato foliage. The *P.infestans* life cycle can be completed in a few days, and as a consequence *P.infestans* infection is explosive. Under ideal conditions, *P.infestans* could increase in population by a factor of 300,000 every 4 days (Fry, 2007). For an efficient potato late blight control is crucial the identification of potato infection with *P.infestans* in due time, before the appearance of the symptoms. That could be done by PCR specific markers for *P.infestans* DNA. RAPD molecular markers could be beneficial for revealing of genetic variability of *P.infestans* accessions (Botez et al., 2004) but is completely inoperative for potato late blight diagnosis. In our researches we have tried to converse *P.infestans* RAPD markers into more specific PCR markers, namely SCAR markers (Sequence Characterized Amplified Region) useful for an operative identification of *P.infestans*.

MATERIAL AND METHODS

Ten accessions of *P.infestans* were isolated on rye medium with antibiotics, from different potato varieties and from different counties of Romania, and were symbolized as: 77, 79, 83, 88, 89, 68, 72, 73, 91, 98. After DNA isolation by Roger and Benedich (1994) protocol, several decamer primers were used for DNA amplification by Maufrand et al., (1995) program of amplification. The amplification products were separated by electrophoresis in 1.4% agarose gel and revealed in UV light. DNA from some polymorphic and monomorphic bands were purified, using Gene Elute Agarose Spin Columns, and cloned into JM-109 and GM2163 *E.coli* strains, using pGEM T Easy Vector Sistem II and GeneJET

PCR Cloning Kit. The second system is a positive selection system for PCR or RAPD amplification products. The pJET1 cloning vector contains the gene occurring a site for a restriction endonuclease, the respective gene is lethal for *E.coli* strains commonly used for cloning. Using specific primers of the vector, the cloned bands were amplified and separated by electrophoresis in agarose gel. Microsynth Company, in the Switzerland laboratory for DNA Sequencing and DNA/RNA Synthesis, sequenced the cloned bands. Relying on nucleotide sequences, there were designed sixteen pairs of primers. The designed primers were tested for amplification of DNA isolated from different accessions of *P.infestans* and from the other species like *E.coli* (plasmidial DNA), wheat, vine, maize and potato.

RESULTS AND DISCUSSIONS

The RAPD amplification products, separated in agarose gel, show a significant variability concerning the polymorphism degree in relation with the primer used and the compared accessions. Thus with primer OPC-9 only two accessions were polymorphic (Fig.1a) while with the primer OPC-20 several accessions were polymorphic (Fig.1b).

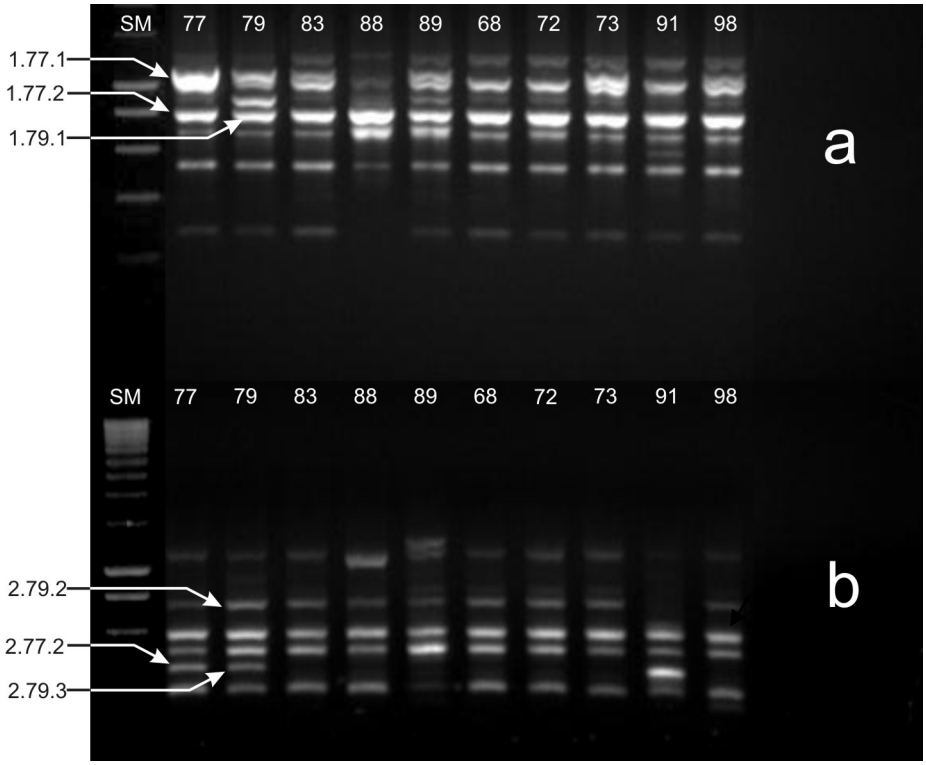

Fig.1 RAPD amplification products obtained from ten *P.infestans* accessions with OPC-9 primer (a) and OPC-20 primer (b). SM-Smart ladder.

Nevertheless, the built dendrogram for the ten *P.infestans* accessions shows a relatively low polymorphism (Fig.2).

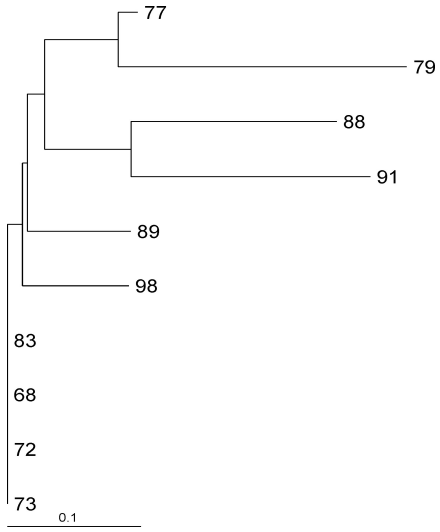

Fig.2 The dendrogram of ten *P.infestans* accessions built with the two primers (OPC-9 and OPC-20)

The amplification products of the cloned sequences, obtained with specific primer of the vector, are presented in Table 1, comparative with original sequences, isolated from the agarose gel, sequences that were cloned. The symbol for the original sequence represents current number of the primer (1 for OPC-9 and 2 for OPC-20), accession and the order number of the sequence isolated from the gel. For PCR amplification there were isolated plasmidial DNA from two host white bacterial colonies, with inserted sequences (Fig.3). The bacterial colonies without insert were blue. Usually the amplification products were almost of the same size with the RAPD original sequences. Also usually the amplification products isolated from the two bacterial colonies were of the same size. However, some times, like in 12G1 and 12G2, or in 13G1 and 13G2, the size was a little different, as we also can see in the figure number 4. Probably isolated DNA from the gel contains several sequences of a little different size.

Table 1

The amplification products of the cloned sequences isolated from two host bacterial colony

RAPD original sequences			Host bacterial colony	Primer used	Amplification products	
Symbol	Type	Size (bp)			Symbol	Size (bp)
1.77.1	Monomorphic	1060	1	OPC-9	11G1	1068
			2		11G2	1069
1.77.2	Monomorphic	775	1		12G1	742
			2		12G2	860
1.79.1	Monomorphic	769	1		13G1	850
			2		13G2	743
2.77.2	Polymorphic	440	1	OPC-20	14G1	500
			2		14G2	500
2.79.2	Monomorphic	740	1		15G1	739
			2		15G2	740
2.79.3	Polymorphic	447	1		16G1	500
			2		16G2	500

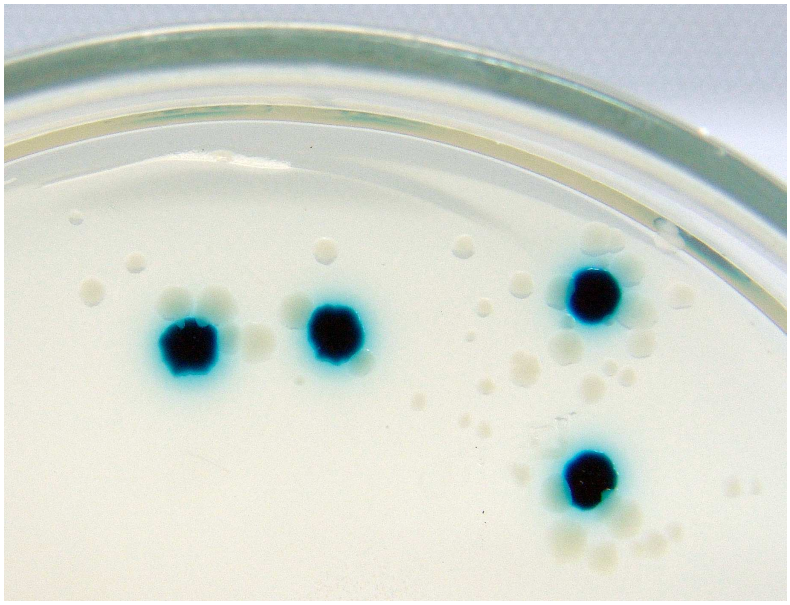

Fig.3 The white and blue colonies, with and without 2.79.3 RAPD polymorphic sequence inserted, cloned into JM 109 *E.coli* strain by pGMT Easy Vector System

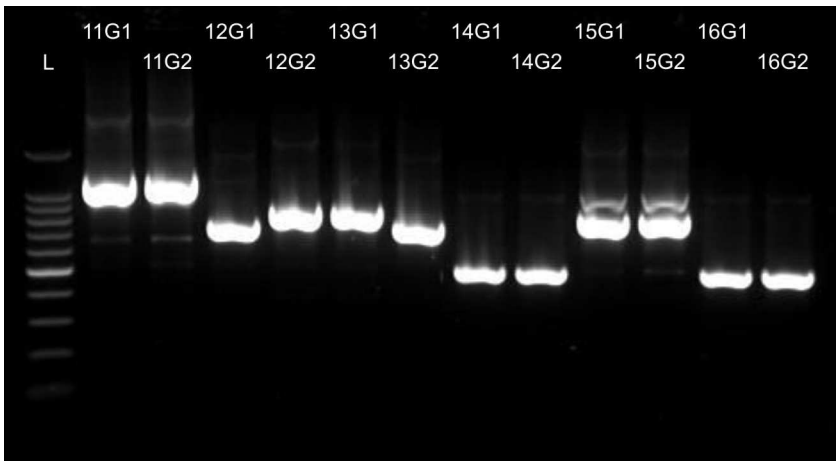

Fig.4 Amplification products obtained from the cloned sequences with specific primers of the vector. L-100 bp ladder.

Six cloned sequences were sequenced and the score of resemblance was established (table 2).

Sometimes, the same size cloned sequences, isolated from the two bacterial colonies, like in 11G1/11G2 or in 15G1/15G2, has attained 100 level of resemblance, showing genetically identity of these sequences. Other times the same size of the cloned sequences has attained almost one hundred level of resemblance, showing a good resemblance, like in 14G1/14G2 or 16G1/16G2.

Table 2

Resemblance scores of cloned sequences isolated from every two host bacterial colonies

RAPD original sequences	Cloned sequences isolated from colony number 1		Cloned sequences isolated from colony number 2		Score
	Symbol	Size (bp)	Symbol	Size (bp)	
1.77.1	11G1	1068	11G2	1069	100
1.77.2	12G1	742	12G2	860	5
1.79.1	13G1	850	13G2	743	2
2.77.2	14G1	500	14G2	500	97
2.79.2	15G1	739	15G2	740	100
2.79.3	16G1	500	16G2	500	99

We have to emphasize that, some times, the two cloned sequences of the same size (14G1 and 16G1), originated from different RAPD polymorphic sequences (2.77.2 and 2.79.3), has 99 resemblance score, indicating a good genetic similarity of the two accessions for that sequences. Other times the two cloned sequences of the same size (12G1 and 15G1), originated from different RAPD polymorphic sequences (1.77.2 and 2.79.2), has 2 resemblance score, indicating a genetic diversity of the two accessions for that sequences (data not shown in this table).

Relying on nucleotide sequences, for every different size of the cloned sequences there have been designed five pairs of primers. Sixteen designed pairs of primers have been synthesised and tested for DNA amplification isolated from different accessions of *P.infestans* or from other species like *E.coli* (plasmidial DNA), wheat, vine, mais and potato. One pair from these (193/194), obtained from the 15G1-cloned sequence (2.79.2 RAPD monomorphic cloned sequence), pair of primer with the following sequence: AGC CCA CAC TGA AAT TAC GC/GGC TAC CGG ATC AGT ACC AA has produced one amplification product only for *P.infestans* (259 bp). So, this pair of primer could be considered specific for *Phytophthora infestans.* Being specific, this pair of primer could be used for a straightforward potato late blight diagnosis from the infected material, and our objective concerning the conversion of *P.infestans* RAPD markers into SCAR markers could be considered accomplished. Nevertheless we have to confirm this specificity, also, in relation with other oomycetes species (*Phytophthora* sp., *Pythium* sp., etc.)

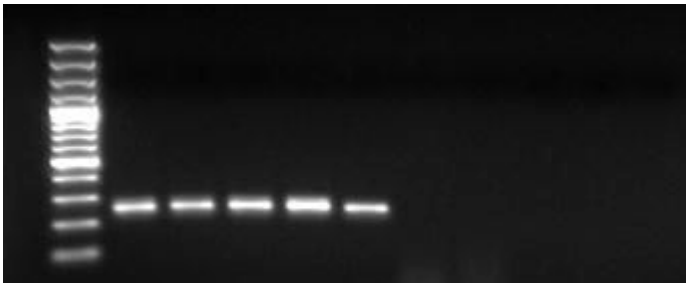

Fig. 5 Amplification products from *P. infestans* (73-91), *E. coli* (E), wheat (W), vine (V), mais (M) and potato (P), resulted with 193-194 built primer pair.

Based on DNA sequences of cloned sequence restriction maps have been realized. We have such a map for 15G1cloned sequence (table 3). These maps could be useful in order to try to differentiate *P.infestans* accessions after amplification product digestion, which was obtained with specific primer pair for *P. infestans*, with different restriction enzyme. For this

aim it would be preferable to use a restriction enzyme that cut in only one position, far from the ends.

Table 3

Restriction map of the cloned 15G1 sequence

Enzyme name	No. cuts	Positions of sites	Recognition sequence
Alu I	3	76, 160, 560	AG/CT
EcoR I	1	319	G/AATTC
Hha I	232	232	GCG/C
Hind III	1	558	A/AGCTT
Hinf I	1	709	G/ANTC
Mse I	3	85, 199, 523	T/TAA
Rsa I	5	315, 481, 584, 619, 632	GT/AC

CONCLUSIONS

The RAPD amplification products, separated in agarose gel, show a significant variability concerning the degree of polymorphism in relation with the primer used and the compared accessions.

The amplification products of the cloned RAPD sequences, obtained with specific primer of the vector, were almost of the same size with the RAPD original sequences.

The same size of the cloned sequences, obtained from different *P.infestans* accessions, is not a guaranty of their genetic identity although it could be.

Usually, the same size cloned sequences, isolated from the two bacterial colonies, shows a good genetic similarity. Some times, the size of cloned sequences, isolated from the two bacterial colonies, was a little different; indicating that isolated DNA from the gel could contains several sequences of almost of same size but genetic different.

One pair of primer designed based on nucleotide sequences, gives 260 bp amplification products only for *P.infestans* accessions and do not for the other species. So, this pair of primer could be considered specific for *Phytophthora infestans* and could be used for a straightforward potato late blight diagnosis from the infected material

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