PCR-based DNA fingerprinting with simple sequences in *Phaseolus*

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Introduction

Minisatellites and simple repetitive sequence motifs (microsatellites) are usually used as hybridization probes for conventional DNA fingerprinting In the present study we used the microsatellites $(GATA)_4$, $(GAGA)_4$, $(GACA)_4$, $(GGTA)_4$, $(GGAT)_4$, $(CAC)_5$, $(CA)_8$ and $(CT)_8$ as single primers in a PCR reaction as described by Meyer et al (1993) with DNA templates from several *Phaseolus* genotypes Amplification with these primers has several advantages over 'classical' DNA fingerprinting techniques and seems to be more reliable than other PCR-based methods, such as random amplified polymorphic DNA (RAPD). The primers $(GACA)_4$ and $(GGAT)_4$ amplified variable fragment sizes, which at least revealed species-specific patterns in different *Phaseolus* species

Material and Methods

The *Phaseolus* genotypes analyzed included *P.vulgaris* var *aborigineus* (NI 573), *P.vulgaris* cv Tendergreen, *P.vulgaris* cv Kentucky Wonder, *P.coccineus* (NI 819), *P.coccineus* (NI 132), *P.lunatus* var *silvester* (NI 583), *P.lunatus* var *lunatus* cvgrp Big Lima (NI 018) and *P.glabellus* (NI 1270) Most of the seed material (NI number) was obtained from the *Phaseolus* collection of the Jardin botanique de Meise, Belgium Total plant DNA was isolated according to Zink et al (1994) The synthetic oligonucleotides $(GATA)_4$, $(GAGA)_4$, $(GACA)_4$, $(GGTA)_4$, $(GGAT)_4$, $(CAC)_5$, $(CA)_8$ and $(CT)_8$, respectivly, were used as single primers in the PCR reaction based on the method of Meyer et al. (1993) Amplification reaction was performed with volumes of 30 µl containing 25-30 ng of total DNA, 1 6 mM MgCl, 200 µM of each of the dNTPs, 2 U of Amplitaq DNA polymerase (Perkin-Elmer Cetus), under the recommended buffer conditions, overlaid with 40 µl of mineral oil PCR was performed for 35 cycles in a DNA thermal cycler (Perkin-Elmer Cetus, model 480) under following parameters 35 sec denaturation at 94°C, 38 sec annealing at (Tm-5°C), and 38 sec primer extension at 72°C, followed by a final extension step for 6 min at 72°C Amplification products were analyzed by electrophoresis in 1 4% agarose gels and detected by staining in ethidium bromide under UV light

Results and Discussion

The high degree of DNA polymorphism detected by conventional multilocus probes in DNA fingerprinting was combined with the technical simplicity of the PCR method The advantages of this technique over 'classical' DNA fingerprinting are: only a small amount of DNA is needed for analysis, the speed of the method, and the possible application to large scale experiments The advantage over the RAPD technique rests on the high reproducibility of the PCR fingerprinting procedure with microsatellites To test the reproducibility, the amplification reactions were repeated three times The results were identical with regard to the number of amplified fragments and their mobility, but variation in the intensity of amplified fragments sometimes occurred.

The results show that the number of bands obtained by PCR fingerprinting depends on the primer used The motifs $(CAC)_5$, $(CA)_8$ and $(CT)_8$, for example, led to so many bands that a smear emerged after staining with ethidium bromide This could be due to the higher frequency of dinucleotide or trinucleotide repetitive sequences in comparison to tetranucleotide repeats

The simple sequence $(GATA)_4$ yielded a distinctive fragment pattern at the species level. However, the fragment profile was not reproducible and primer artefacts were detected This phenomenon can be explained by the very low GC% content of the primer sequence.
PCR fingerprint priming with $(GAGA)_4$ and $(GGTA)_4$ also revealed a smear However, a few distinctive bands were detectable
At the species level, the PCR fingerprint patterns obtained with the primers $(GACA)_4$ and $(GGAT)_4$ clearly differed from each other in *P.vulgaris*, *P.coccineus*, *P.lunatus* and *P.glabellus* No intraspecific variation was revealed with the $(GGAT)_4$ motif in *P.vulgaris*, *P.coccineus* and *P.lunatus* genotypes
In contrast, two group specific patterns corresponding to the Middle American and Andean grouping of the *P.vulgaris* genotypes were found using $(GACA)_4$ Furthermore, with this motif intraspecific variation was observed in the *P.coccineus* and *P.lunatus* genotypes
The PCR fingerprinting technique seems to be less sensitive than conventional DNA fingerprinting For example, DNA fingerprinting analysis with microsatellites by Hamann et al (1995) could distinguish *Phaseolus* gene pools, cultivars, and in some cases, differences among individuals However, PCR fingerprinting might be suitable for interspecific comparison and hence be a useful tool to determine the phylogenetic relationship of *Phaseolus* species Further, it has to be tested if the same sensitivity as with 'classical' DNA fingerprinting could be obtained with different primer sequences, pairwise primer combinations, or endonuclease restricted template DNA

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