Specific amplification of the phaseolin-genes in *Phaseolus vulgaris*, *Phaseolus coccineus* and *Phaseolus lunatus* by the polymerase chain reaction (PCR).

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**Introduction**

Studies on the genetic diversity in *Phaseolus vulgaris* often use the properties of the storage protein phaseolin as a molecular and evolutionary marker (Gepts, 1990). Phaseolin is composed of α-, β-, and γ-polypeptides which differ in their molecular weights. The genes for the β- and the α-polypeptide were identified and sequenced (Slightom et al., 1981; 1985; Anthony et al., 1990). It was found that the α- and β-phaseolin genes exhibit a great homology and differ from each other, mainly in the presence of one or both direct repeats found in α-phaseolin genes. Additional studies revealed that the phaseolin genes are organized in a multigene family with 7-8 genes per haploid genome (Talbot et al., 1984). All these data refer to *Ph. vulgaris*. So far no information concerning the phaseolin genes in other species, or wild forms, is available.

We present herewith an approach that allows to study the phaseolin genes at the DNA level in *Ph. vulgaris* and two other species, employing the polymerase chain reaction (PCR; Saiki et al., 1985). This technique allows to amplify a sequence of interest with specific primers in vitro (the use of this technique for fingerprinting *Ph. vulgaris*, was recently discussed by Stockton et al., 1992).

**Materials and methods**

Total DNA was isolated from leaves of *Ph. vulgaris* cv. Tendergreen, *Ph. coccineus* cv. Preisgewinner and *Ph. lunatus* cv. Fordhook bush #242. The primers were selected on the basis of the phaseolin gene sequences known for *Ph. vulgaris* (β- and α-phaseolin genes from cv. Tendergreen: Slightom et. al 1981; 1985; α-phaseolin gene from cv. Sanilac: Anthony et. al 1990). Their annealing position lies in exon 1 and exon 6 respectively, thus allowing to amplify a great part of the phaseolin genes. PCR was performed using AmpliTaq (Perkin Elmer). Negative controls lacking either DNA, or primers, or the Taq polymerase, were included to check whether detectable fragments are produced in these cases.

After PCR, the success of the amplification was controlled by agarose gel electrophoresis. For a brief screening of sequence variations, restriction enzymes were selected which cut once in the known sequences (HaeII, HpaII, SpeI, BglII, BsaJI, BsmAI, XbaI, EcoRV and PstI). The only exception was PstI, which has an additional recognition site in intron 2 of the α-phaseolin gene sequence as given by Anthony et al. (1990).

Fragment length determinations of the entire fragments and the restriction redigests were performed on high resolution agarose gels consisting of 2% or 3% Nusieve (3:1; FMC).

To prove the amplification of phaseolin genes, a high stringency Southern hybridization (at least 10min 68°C, 0.1xSSC) was performed with a recombinant β-phaseolin gene probe (clone AG-pPVP 3.0, a kind gift of Dr. J.L. Slightom, Madison, WI).

**Results and discussion**

**Generation of phaseolin gene specific fragments by PCR**

In the three *Ph. vulgaris* species investigated, all amplifications yielded a specific fragment without any detectable artifacts. All controls were negative, indicating that the products were only formed in a complete PCR reaction. The high stringency hybridization showed a strong signal confirming the presence of sequences highly homologous to the phaseolin genes. Nevertheless, the signal of the amplified fragment from *Ph. lunatus* was slightly, but significantly weaker.

**Size of amplification products**

In case of *Ph. vulgaris* and *Ph. coccineus* the fragments generated were of the size, as expected from
the known sequences of *Ph. vulgaris*. In contrast, the fragment sizes of the amplified products in *Ph. lunatus* were significantly larger. Additionally, in *Ph. vulgaris*, compared to *Ph. coccineus*, a slight distribution of not distinctly resolveable fragments were seen. These could be possibly the consequence of two direct repeats (a total of 52bp), known to be respectively present and absent in the α- and the β-phaseolin genes.

**Restriction site analysis**

*Ph. vulgaris*

All enzymes generated fragments which correspond with those expected from the known sequences for the α- and β-phaseolin genes. Using PstI, the presence of the α-phaseolin gene, having an extra recognition site in intron 2 (Anthony et al., 1990), could be shown.

*Ph. coccineus*

In this species all restrictions revealed evidence for an organization of the phaseolin genes different from that in *Ph. vulgaris* (with α- and β-forms), as no extra fragments, resulting from the presence of one or both direct repeats, were detectable. This result could be interpreted as an indication for a more uniform sequence of the phaseolin genes, or that only one phaseolin gene is present in *Ph. coccineus*.

*Ph. lunatus*

Here only the enzymes XbaI, PstI, BsmAI, SpeI and BglII produced two fragments as expected for a single cutting site in the gene. The fragment sizes were different from those in *Ph. vulgaris*, according to the larger fragment amplified. All other enzymes produced more, or no (HpaII), detectable restriction fragments. These results are in agreement with the fact that *Ph. lunatus* is more distantly related to *Ph. vulgaris* than, e.g., *Ph. coccineus*.

**Summary and Conclusions**

1. By using PCR it is possible to amplify the phaseolin genes selectively, and with high specificity, out of the complex mixture of DNA fragments present in isolated total DNA.
2. The species of investigation can be as different as *Ph. vulgaris* and *Ph. lunatus*.
3. In the three species investigated, length polymorphisms for the phaseolin genes were found.
4. By the use of the restriction enzyme PstI it was possible, to clearly confirm in the amplification products of *Ph. vulgaris*, the presence of at least one α-phaseolin gene (of the phaseolin multigene family), exhibiting an additional site for this restriction enzyme.
5. This approach to study the phaseolin genes seems to be promising as it can increase information at the DNA level, supplementary to the knowledge received at the level of the phaseolin polypeptides. PCR enables the comparative study of these genes for length polymorphisms restriction site variants and facilitates sequencing.

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**Literature cited**