Identification of synthetic oligonucleotide probes revealing polymorphism in *Phaseolus lunatus* L.

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Introduction

The primary gene pool of Lima bean (*Phaseolus lunatus* L.), one of the main cultivated species of *Phaseolus*, may be divided into two morphogroups, a Mesoamerican and an Andean one. Each group comprises both wild and cultivated forms. Recently, phylogenetic studies on *P. lunatus* supported an Andean origin of the species (Fofana et al., 1999; Maquet et al., 1999). Two main independent domestication events gave rise to the Andean and Mesoamerican races, with specific morphological characters, seed storage protein and allozyme patterns (Gutiérrez Salgado et al., 1995).

Microsatellites, or simple sequence repeats (SSRs), are hypervariable genomic regions in which one or few motifs of a small number of base pairs are highly repeated. Their presence and abundance in the genome of *P. vulgaris* has been shown by PCR amplification (Yu et al., 1999). The use of synthetic oligonucleotides as probes for microsatellites in genomic DNA has proven useful in creating fingerprints (Hamann et al., 1995) often specific to single varieties or even individuals. Presently, genetic variability and relationships among cultivated and wild forms of Lima bean are being investigated by microsatellite fingerprinting.

Materials

Accessions belonging to either the Mesoamerican or the Andean gene pool were chosen according to their protein pattern and, for wild material, to the geographical range of the species. Seeds were obtained from CIAT, Cali, Colombia or from IPK, Gatersleben, Germany. Young leaves were collected from single plants of the 24 accessions, including 12 wild forms and 12 landraces. Genomic DNA, isolated according to the Dellaporta et al., (1983) method and further purified using S-200 columns (Pharmacia), was digested with different restriction enzymes (*Alu* I, *Dra* I, *Eco* RI, *Hae* III, *Hind* III, *Mbo* I, *Msp* I, *Rsa* I, *Taq* I), electrophoretically separated on 1% agarose gels, and transferred onto nylon membranes. Twelve digoxigenated synthetic oligonucleotides: *(AT)*₈, *(CA)*₈, *(AAC)*₅, *(AAT)*₅, *(CAT)*₅, *(GTG)*₅, *(AAAC)*₄, *(AATG)*₄, *(AGGT)*₄, *(GACA)*₄, *(GATA)*₄, *(GGAT)*₄, were used as probes and chemiluminescent signal detection was performed with CDP-Star (Amersham).

Results and discussion

In a first set of experiments, we investigated the effectiveness of various combinations of restriction enzyme and digoxigenated synthetic oligonucleotide probe. All the different simple repetitive motifs used as probes were present, although at different levels of abundance, in the genome of Lima bean genome. Some of these synthetic oligonucleotide probes, independently of the restriction enzyme used, produced a strong hybridisation smear, while others, such as *(GACA)*₄, *(GATA)*₄ and *(GGAT)*₄, showed a number of discrete bands ranging between 2 and 21 kb (see figure 1). Generally, the hybridisation pattern within each accession appeared to have a limited variability, although some intra-individual polymorphisms could be observed in the high molecular weight regions. Conversely, highly polymorphic patterns were observed among accessions, each showing a distinct banding pattern.
Simple sequence repeats have proven to be optimal tools to discriminating among either accessions or individuals and to characterizing genotypes.

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Literature cited

Figure 1. Hybridisation patterns of digoxigenated synthetic oligonucleotide (GATA)n as probe to DNA from different P. lunatus genotypes, digested with Hae III. M: DNA size marker in kb