MICROSATELLITE MARKERS FOR COMMON BEAN

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Introduction

Marker-assisted selection (MAS) has been proposed as a useful method in plant breeding as it can help speed up the selection process and reduce the selection costs during the early generations of a breeding program. Cregan et al. (1999) emphasized the importance of the DNA markers not only for assisting selection, but also for genomic analysis, evolutionary studies and gene cloning. Therefore, molecular markers are important to plant breeders as a source of genetic information, as well as for use in the indirect selection of economic traits linked to the marker (Kelly, 1995).

Most of the markers identified in common bean are RAPD and AFLP types, however, new markers such as the microsatellites (SSRs) have gained popularity among breeders and plants geneticists. This class of codominant marker that detects a high level of allelic variation associated with the economical and simple PCR procedure, has become an efficient tool for studies of eukaryotic genes (Panaud et al., 1996). In addition to these traits, they are the preferred markers to assist in breeding several crops because they are randomly distributed in the genome and they are easily reproducible (Rallo et al., 2000). The use of these efficient markers in common bean has been limited by the lack of available primers developed for the species. In this work, microsatellite markers were developed for common bean, using bacteria artificial chromosome (BAC) clones.

Materials and Methods

Four BAC clones chosen from the genomic library of the bean cultivar Sprite (Van Houten and McKenzie, 19??; Melotto and Kelly, 2002) and made available through the bean breeding program at Michigan State University were subcloned, using three different combinations of restriction enzymes: *AluI/Hpa*I, *RsaI/Nae*I, and *Hinc*II/*Xmn*I. The small insert library was hybridized with three SSR probes. After two hybridization cycles, the positive clones were sequenced. The sequences were analyzed for the redundancy and presence of SSR sequences. Specific primers, complementary to the sequences that flank the SSRs, were designed and tested in the original BAC clones and in ten bean cultivars. Six cultivars selected; Rudá, BAT 332, Cornell 49-242, Mexico 54, MAR-2, and AND 277 came from the common bean breeding program for resistance to angular leaf spot, developed by BIOAGRO-UFV, Viçosa, Brazil; BAT 93 and JaloEEP558, the parents of the consensus bean map (Freyre et al., 1998) were chosen; and Black Magic, and SEL1308 the parents of mapping population developed at MSU to study anthracnose resistance in common bean (Melotto and Kelly, 2001) were chosen.

Results and Discussion

The subcloned fragments were hybridized with the microsatellite probes, $(AT)_{15}$, $(CT)_{15}$ and $(ATT)_{10}$. These oligonucleotides were chosen because they are the most frequent SSRs in higher plant genomes (Morgante and Olivieri, 1993; McCouch et al., 1997); and the same AT/TA and CT/AG, repetitions were reported as the most common in the bean species (Yu et al.

1998, 1999). From 890 colonies analyzed, 8% or 72 hybridized positively. When the clones were sequenced, 27 showed microsatellite sequences (Table 1), and some exhibited more than one microsatellite. Primers were designed and tested, but three did not generate any product, while five amplified several bands. Twenty-one primers pairs amplified a clearly defined and unique band, of which 15 were polymorphic and six were monomorphic. The allele numbers per locus ranged from one to six, showing a high degree of polymorphism for these markers. According to Weber (1990), the number of alleles in a microsatellite is usually correlated with the number of repetitions they possess; in general, a higher number of repetitions leads to higher polymorphism. However, some primers observed in this research had SSRs with only three repetitions and amplified two alleles, whereas others with 5, 6 and 11 repetitions produced a monomorphic band. These observations suggest the lack of any correlation between the number of repetitive units and the number of detected alleles in common bean. A similar lack of correlation was also reported by Panaud et al. (1996), Yu et al. (1999), and Rallo et al. (2000).

The described procedure was shown to be efficient for the development of microsatellite markers in common bean. The procedure was relatively fast, with lower associated costs and labor, compared to other methods that involve the construction of a genomic library. In this study, however, the developed primers can only be used to analyze the small area of the genome that contained the BAC clones. These SSR markers will be highly useful to saturate the specific area of the genome where major disease resistance traits are known to reside (Melotto and Kelly, 2001). This group of new microsatellite markers, combined with the other available markers, should also provide an important tool in the breeding and genetic of common bean. The future identification of other markers utilizing other libraries should be performed, so that the entire bean genome can be saturated with SSR markers.

Table 1. Sequenced clones results

Sequenced clones	62
Clones without insert	6
Clones with *Escherichia coli* DNA	3
Redundant sequences	4
Problems with the sequencing	6
Sequences with no microsatellites	16
Sequences with microsatellites	**27**

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