DEVELOPMENT, TRANSFERABILITY AND MAPPING OF MICROSATELLITE MARKERS ON A REFERENCE BAT93 X JALOEEP558 POPULATION

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INTRODUCTION

Cultivated common bean (*Phaseolus vulgaris*) is a globally important crop. The advanced genomic studies of common bean can be successfully accelerated due to the development of new molecular tolls providing an opportunity for breeders to accelerate the development of varieties with valuable agricultural traits. The reduced genome size and the increasing pool of genetic resources is clearly a promising field with high potential to provide significant advances in research methodology useful for geneticists and breeders related to identification and elucidation of target genes. Codominant markers, such as microsatellites, are better suited for genome mapping because they are more informative and easily transferable. Microsatellite markers begun to be integrated into common bean linkage maps by Blair et al. (2003, 2006), resulting in a linkage map based exclusively on microsatellite markers mapped in the BJ population (Grisi et al. 2007) and, more recently, a new expanded version of the core linkage map also using the BJ population was released, which included markers with putative gene function (Hanai et al. 2009). The main objectives of the present work was: 1) to develop and to make available a set of SSR derived from express sequences (EST) of *Phaseolus vulgaris* obtained from the GenBank; (2) to genetically characterize a group of EST-SSRs and genomic SSR markers, 4) to examine the transferability of SSR markers among species of the Leguminosae family, 5) to integrate a set of new microsatellite markers into the core map for Bat93 x Jalo EEP558 population.

MATHERIAL AND METHODS

The EST sequences were obtained from the "*Phaseolus vulgaris* EST Project site" (**http://www.ccg.unam.mx/phaseolusest/**) and the Primer3 software used for primer design. The total number of 377 was synthesized, adjusted for PCR amplification and screened for polymorphism between the genitors BAT93 and JALO EEP558. The polymorphic markers were genotyped in a progeny consisting of 74 recombinant inbred lines (RIL) in the F_8 generation. The whole set of new segregant markers was integrated into a framework map composed of 123 SSRs markers, previously mapped in BJ population (Grisi et al., 2007). A total of 167 SSRs, being 107 previously published and 60 newly EST-SSRs, were selected for the analysis of transferability across 10 species of the Legumes genus, representative of four important tribes and one subfamilies of the Leguminosae family.

RESULTS

According to the criteria for the SSR containing sequence identification, a total of 9583 valid ESTs were screened for the presence of useful SSR sequences and 4764 sequences containing SSRs were identified. In the evaluation, out of the 377 EST-SSRs from *P. vulgaris*, 302 (80%) showed scorable

amplified product. while 24 generated non-specific products and 72 failed to amplify. Thus, a total of 315 markers were screened for the polymorphism in the BJ population, followed by the linkage analysis of the segregant markers. To access the transferability of SSR loci across Legumes species, the cross amplification of 167 primers (65 genic and 102 genomic- SSR) against 20 genotypes representing 10 species of the Leguminosae family was performed (*Medicago sativa, Phaseolus lunatus, Phaseolus coccineus, Phaseolus acutifolius, Vigna mungo, Vigna angularis, Vigna unguiculata, Glycine max, Arachis hypogaea* and *Dipteryx alata)*. From the 65 genic SSRs, a total of 61 (94%) amplified across, at least, one species and only four (6%) were specie specific. For the 102 markers tested derived from genomic libraries, 76 (75%) amplified across, at least, one species and 26 (27%) failed to produce an amplification product across the species. The ratio of transferable markers among the species ranged from 119 (71%) to three (1.8%), respectively, for *P. Acutifolius* and *A. hypogaea*, respectively, with a mean of 32% of cross amplified loci. As expected, the high index of interspecific cross amplification were observed for species within the genus *Phaseolus* (64%), followed for *Vigna* (26%), *Glycine* (20%), *Medicago* (10%) and *Dipterix* (6%). For the whole set of 167 SSRs tested, the mean PIC values was 0.50, from the 68 genomic SSRs the average value was 0.53 and among the EST- SSRs the mean PIC value was estimated in 0.47. Of the 315 newly SSRs screened for the polymorphism, 76 segregated in the BJ population, of which 72 were EST-SSRs and four anonymous SSRs. The integration of the SSRs into the reference linkage based exclusively in SSRs resulted in a dataset of 199 polymorphic markers, being 117 genomic SSRs and 82 EST-SSRs. Of these, a total of 180 (90%) markers was mapped and distributed in 13 chromosomes. The distribution of the EST-SSRs appeared to be relatively random and dispersed throughout the *Phaseolus* genome, of which every linkage group contained more than one EST-SSR marker. The comparative analysis, based on common SSR markers, performed between the current based SSR map and the based SSR map previously developed by Grisi et al. (2007) showed that all SSR markers (99%), but one (BM202), maintained their position in the same linkage group. A considerable degree of homology was observed in terms of marker order conservation (78%). Based on the present work a broad set of useful SSR markers for common bean derived from public EST databank was developed. Not surprising, the present results indicated that EST-SSRs are more transferable across the Legumes species than are anonymous SSRs and the level of EST-SSR polymorphism (0.47) was slight lower than that with SSR derived form genomic libraries. Despite the reduced level of polymorphism rates of the EST-SSR, these markers were very useful for genetic mapping of the BJ populations helping to increase the map coverage in the *Phaseolus* genome.

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