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Determination of the population structure of common bean (*Phaseolus vulgaris* L.) accessions using lipoxygenase and resistance gene analog markers

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ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is an important food legume throughout the world. Because of the conservation across different plant species, it is possible to evaluate the degree of genetic diversity in the common bean using gene-based marker techniques. The lipoxygenase (LOX) and resistance gene analog (RGA) genes play an important role in the response to biotic and abiotic stresses. Eighty-six common bean accessions were genotyped using gene-based LOX and RGA markers. The total number of polymorphic bands ranged from 193 for LOX to 17 for RGA markers. We detected considerable diversity with a mean of 8.7 alleles per primer for the LOX analysis. For the RGA markers, the number of alleles per polymorphic locus varied from 1 to 4 with an average allele number of 2.8. The genetic similarity between the accessions based on the LOX and RGA markers ranged from 0.12 to 0.55. Using STRUCTURE, 3 groups were revealed among the accessions. The results of this study should provide valuable information for future studies on the genetic diversity of common bean accessions and for association mapping studies examining the relationships between the genotypic and phenotypic traits related to the stress response.

1. Introduction

Phaseolus vulgaris or the common bean is an important food legume in many countries around the world because it has a high protein level and is a good source of important vitamins and minerals. The common bean represents a principal source of daily protein for direct human consumption (Singh, 2000). According to archaeological observations, the common bean originated in Peru and the southwestern United States (Gepts et al., 1988). Currently, it has become the most widely cultivated legume in many developing countries, particularly in Africa, due to its nutritive components (Broughton et al., 2003). Globally, the annual production of green and dry beans is 17 Million tons (FAO, 2010). Common bean production is almost twice that of the second most important legume, the chickpea (*Cicer arietinum* L.) (Gepts et al., 2008). The genus *Phaseolus* contains approximately 70 species, providing a large genetic resource (Kwak and Gepts, 2009). Therefore, it is important to understand the genetic diversity of the common bean germplasm. The common bean is a diploid ($2n = 2x = 22$) with a genome size of 588

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mega base pairs (Mbp) and it is a predominantly self-crossing species (Arumuganthan and Earle, 1991). Despite the medium-sized genome, the common bean is a model organism for other legumes.

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12, LOX) enzymes are non-heme iron-containing enzymes that catalyze the conversion of polyunsaturated fatty acids (PUFAs) to hydroperoxy fatty acids in plants, animals, and microorganisms (Prigge et al., 1996). LOXs are present in the seeds, seedlings, and leaves of many plant species, particularly legumes (Hessler et al., 2002; Siedow, 1991). For instance, the LOX protein accounts for up to 2% of the total protein content of soybean seeds (Loiseau et al., 2001). The two most common substrates for LOX are linoleic acid and linolenic acid in higher plants which serve as precursors of several metabolites, including the well-known hormone jasmonic acid (Feussner and Wasternack, 2002). The LOX enzyme plays important roles in several processes including the response to pathogens and wounding, nitrogen storage, senescence, mobilization of stored lipids during germination and the biosynthesis of regulatory molecules (Loiseau et al., 2001).

The LOX genes have been shown to be conserved across plant and mammalian genomes (De La Fuente et al., 2013) and plant LOXs can be grouped into two gene subfamilies depending on their sequence similarity. The first subfamily is the LOX1-type LOXs, which have been identified in several plants including potato (Geerts et al., 1994) and cucumber (Matsui et al., 2006). The other subfamily, called type 2, has been characterized in plants such as tomato (Heitz et al., 1997). LOX1-type genes have high sequence similarity (75%) while LOX2-type genes show only moderate sequence similarity (35%) (Liavonchanka and Feussner, 2006). For example, 15-LOX from soybean shows 25% identity with the mammalian 15-LOX enzymes and the two human 15-LOXs share 35% identity with each other. On the other hand, distinct subgroups among close species exhibit 70–95% sequence identity (Brash, 1999). Additionally, several studies have examined the similarity of LOX enzymes in the common bean. LOX1 (Eiben and Slusarenko, 1994) and pLOX3 (Meier et al., 1993) show 84.2% and 72.7% homology, respectively, to PvLOX2 in the common bean genome (Porta et al., 1999).

Plant disease-resistance (R) genes are classified based on their protein structures (Dangl and Jones, 2001). Approximately 75% of the R genes, such as *Arabidopsis* RPM1 (Grant et al., 1995) and pepper CaMi (Chen et al., 2007) have been identified in the plant genome. These genes are crucial because of the complex mechanisms of resistance and the interactions involved in pathogen recognition (Selvaraj et al., 2011). Resistance gene analog (RGA) markers are designed using the conserved motifs of the nucleotide-binding site (NBS), the leucine-rich repeat (LRR) and the protein kinase domain of genes that are the most likely to be target genes for disease resistance (Chen et al., 1998). RGA primers have several advantages over arbitrary markers: (i) these markers are useful for different plant species because of the use of conserved sequences and (ii) they are highly reproducible due to the use of longer primers (Naik et al., 2006). Several RGA markers have been successfully used in many studies involving the genetic mapping of resistance genes (Kanazin et al., 1996; Maleki et al., 2003; Zhang et al., 2002) and investigation of the diversity and genetic variation of rice (Ren et al., 2013), wheat (Dong et al., 2009) and common bean (Mutlu et al., 2006).

The main objectives of this work were to detect genetic diversity among the common bean population using the conserved LOX gene and RGA primers as resistance gene base markers. To the best of our knowledge, this is the first LOX- and RGA-based genetic diversity study in the common bean.

2. Materials and methods

2.1. Plant material and extraction of DNA

Eighty-six accessions of the common bean were used in this study are listed in selected to represent the most important accessions in Table 1. The genomic DNA was extracted from young leaf tissues of seedlings for each accession. A hundred mg of leaf tissue was ground to a powder in liquid nitrogen using Tissue Lyser (Technogen Co. Izmir, Turkey). The DNA was extracted using the DNeasy Plant Mini Kit (Qiagen #69106). The quality of the DNA was confirmed by electrophoresis in a 0.8% agarose gel, and the DNA concentration was measured using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The final DNA concentration was adjusted to 100 ng/μL for LOX and 30 ng/μL for RGA analysis, and the diluted DNA was stored at −20 °C for the PCR reactions.

2.2. LOX analysis

The twenty-four LOX primers that were obtained from Liu et al. (2011) and used for genotyping are listed in Table 2. The LOX primers were modified by the addition of an M13 tail (CACGACGTTGTAAAACGAC) to the 5′ end of the forward primer labeled with two different fluorescent dyes, IRD 700 and IRD 800 (Maccaferri et al., 2008). The reverse primers were unlabeled. The amplified products were size separated by 8% polyacrylamide gel electrophoresis in 1× TBE (Tris-borate-EDTA) buffer under the conditions of 1500 V and 40 mA in a LiCor 4300s DNA Analyzer. Image processing to evaluate the fragments was performed using SAGA software (LiCOR Biosciences, Lincoln, NE, USA).

2.3. RGA analysis

Eight RGA markers were analyzed in this study (Dong et al., 2009) are listed in Table 2. PCR was carried out in a 20 μL reaction volume containing 30 ng/μL genomic DNA, 1 unit of Taq DNA polymerase (Thermo Sci. Co.), 20 mM $MgSO_4$, 10 mM of