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Short communication

Genetic diversity assessment of a set of introduced mung bean accessions (*Vigna radiata* L.)

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ABSTRACT

Genetic resources from other countries or regions play an important role in broadening the genetic background of local breeding varieties. Here we describe observations on the adaptability of mung bean germplasm obtained from the United States Department of Agriculture and their genetic diversity assessment using SSR markers. Several accessions were shown to be mixtures, based on their phenotypes for some characters. Most accessions were able to complete their lifecycles when grown in Beijing, China, making them ideal for crossbreeding without day length control. High diversity was revealed by the SSR markers, with an average of 4.2 alleles per locus and a PIC value of 0.650 per locus. STRUCTURE analysis divided the accessions into six groups. There was no obvious trend of accessions forming groups according to their geographical origin, owing mainly to germplasm exchange and an uneven distribution of accessions. The present results indicate that this germplasm would enrich the local gene pool, and provide information for the further use of germplasm in breeding programs.

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1. Introduction

The mung bean (*Vigna radiata* L.), also known as green gram, is an important pulse crop providing vegetable protein for people throughout Asia [1]. Mung bean is a traditional food in China and is widely grown in monoculture in dry and semi-dry regions, as well as being used as an intercrop throughout much of the country because of its drought tolerance and nitrogen-fixing soil fertilization [2]. Owing to its short lifecycle, mung bean is also used as a post-disaster remedy when crops are destroyed by a natural disaster during their middle growth phase and it is too late to sow them again. However, the production of mung bean now faces novel challenges arising from climate change. The discovery of new genes and the development of varieties with multi-resistance or tolerance to diseases, pests, and extreme

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climates is the best strategy for sustainable production of this crop.

Assessments of phenotypic [3–5] or genotypic [6–8] diversity provide beneficial information for the better use of germplasm collections, which are the basic material for genetic improvement and breeding. Following a country-wide collection during the 1980's, over 3000 accessions of mung bean germplasm were conserved in the national gene bank of China, and this collection is continually being added to [9–11]. However, evaluations of local germplasm have shown that there are few elite genes conferring resistance to biotic and abiotic challenges, with the result that the genetic backgrounds of modern varieties have become gradually narrowed [12]. Previous reports have shown that alien or wild accessions can be of great value in local breeding [13,14]. For instance, mung beans from the Asian Vegetable Research and Development Center (AVRDC) have contributed greatly to increasing the yields of local breeding cultivars in China compared to those at the end of the last century [12,15]. Additionally, a bruchid-resistance gene has been successfully transferred to cultivated genotypes from wild mung beans [16,17]. Thus, the best approach to enriching the local genetic pool is to introduce more germplasm from other countries or genes from wild relatives.

We recently obtained a set of mung bean accessions from the germplasm resources information center of the U.S. Department of Agriculture (USDA), a repository with a large mung bean collection. In the present study, we recorded the seed characters of these accessions, sowed them in Beijing to observe their growth, and evaluated their SSR variation. The main aims were 1) to determine whether the accessions can complete their lifecycle in Beijing and have potential use in cross-breeding without day length control, and 2) to assess the germplasm allelic richness by SSR variations to obtain information for further study.

2. Materials and methods

2.1. Plant materials

A total of 184 mung bean accessions, obtained from the germplasm resources information center of the USDA (http://www.ars-grin.gov/npgs/aboutgrin.html) in 2012, were used in the study. Of these accessions, 178 were originally collected from 22 countries, two were from the Middle East, and four had no passport information (Table S1). After observation of their seed characters (color and testa), the accessions were planted in a greenhouse in early April 2013 in Beijing. Zhonglyu 5, a popular Chinese variety, was used as a control. Owing to the limited number of seeds available, only 10 seeds of each accession were planted, with row and plant spacing set at 50 cm and 12 cm, respectively. An equal weight of fresh leaves from each individual within each accession was collected for DNA extraction.

2.2. Phenotypic observation

In addition to seed characters, four other traits were investigated. Anthocyanidin coloration in the young stem was first observed and used to partially determine the homozygosity of each accession. Growth period, pod length, and seed weight were recorded as well, following Cheng et al. [18]. These three characters provide information directly useful for the application of germplasm in local breeding programs.

2.3. SSR analysis

Genomic DNA was extracted using the CTAB method [19]. Thirty-eight polymorphic SSR primers were used, with 24 from mung bean [20], 12 from adzuki bean [21], and two from common bean (obtained from http://www.ciat.cgiar.org/biotechnology/SSR_table.html). PCR analysis was performed in a 20-μL reaction solution containing 1× PCR buffer, 100 μmol L^{-1} of each dNTP, 0.4 μmol L^{-1} of each primer, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase. Amplification was performed in an EDC-810 thermal cycler (Dongsheng Co. Beijing) with 35 cycles of 94 °C for 30 s, 47 °C for 30 s, and 72 °C for 30 s, followed by a final 5-min extension. The product was separated by 8% SDS-polyacrylamide gel electrophoresis (PAGE), using 0.5× TBE as a buffer at 220 V. The running time of electrophoresis was adjusted according to the expected size of products, usually to 1.0–1.5 h.

2.4. Genetic diversity and STRUCTURE analysis

All SSR loci were scored as 1 if present and 0 if absent for each polymorphic fragment. The observed number of alleles (NA), the polymorphism information content (PIC value) and the expected heterozygosity were calculated using Popgene [22].

STRUCTURE 2.3.4 [23,24] was applied based on multi-loci genotype data to assess the population structure of and the genetic relationships between the accessions. An admixture model and an independent allele frequency model were fitted to analyze data lacking prior population information. These models were run ten times for each number of populations (*K*) (varied from 1 to 10), and the maximum likelihood ratio was used to assign accessions to clusters [25]. Genetic similarity coefficients (GSC) and genetic distances (GD) between accessions were calculated, and UPGMA (unweighted-pair-group method with arithmetic mean) was used for cluster analysis using NTSYS-pc2.10 [26] based on genetic distances.

3. Results and analysis

3.1. Observations on the introduced germplasm

Among the 184 accessions, green seeds accounted for 78% and black seeds for 13%. Brown, yellow and dotted seed coats accounted for 3%, 4%, and 3%, respectively. Seed testa with glossy surfaces accounted for 76% and those with dull surfaces for 22%. Three accessions were assumed to be mixed, because both glossy and dull seed testa were observed within one accession; of these, one was from India (PI 346316), one was from Pakistan (PI 268412), and the other had no passport information (PI363239). Thus, only seeds that had a prevalent seed testa were planted for the three accessions.

All of the accessions successfully emerged within a week after sowing. Eleven of them were observed to be genotype mixtures based on the anthocyanidin coloration of caulicles

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