Genetic Studies of the Asian Subgenus *Ceratotropis**

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Introduction

Ceratotropis is the most important subgenus under the genus *Vigna* and it comprises of five major domesticated crops such as mungbean, black gram, adzuki bean, rice bean and moth bean. The mungbean, in particular, was first introduced to Malaysia in 1917 (Kassim *et al.*, 1978). Since then it has been a forgotten crop. In the early nineties field surveys were conducted to collect wild samples of mungbean from various locations of the Peninsular Malaysia (Bujang *et al.*, 1994). The wild species are especially important since desirable genes from it could be incorporated into cultivated forms of the subgenus to enhance its commercial value. Very little is known about the genetics of Asian Ceratotropis especially in its breeding systems, cytogenetics, nodulation habits, and general structure of populations and its phylogenetic relationships. Lack of this information would lead to costly errors made by plant breeders in their breeding programs such as loss of genetic variability or diversity, which cannot be regenerated. In this study, we have used protein level markers as well as DNA based markers to genetically evaluate the mungbean.

Materials and Methods

A total of 183 accessions of wild *Vigna* seeds sampled from Pahang, Selangor and Johor were used in the isozyme analysis. Extracts of the seeds were subjected to vertical polyacrylamide gel electrophoresis at 4°C and the gels stained for the different enzymes following the protocol of Quah et al. and Ishak (1998). The data were analysed using BIOSYS-1 computer package. As for the DNA based analyses, DNA was extracted from seeds using the Clontech Laboratories DNA extraction kit. A total of 12 varieties and varietal crosses of domesticated and wild mungbean were used in the Random Amplified Polymorphic DNA (RAPD) analysis following the protocol of Kumar *et al.* (1998). The PCR product was electrophoresed on a 2% agarose gel and stained with ethidium bromide. The data collected were analysed using RAPDistance Package and NTSYS computer software programmes. For the microsatellite analysis, 12 pairs of primers originally designed to amplify the *Phaseolus* genome was used to amplify the mungbean DNA. The PCR product was electrophoresed on a 4% Nuseive 3:1 agarose gel and stained with ethidium bromide. To develop microsatellites for *Vigna*, PCR products from the RAPD and Randomly Amplified Microsatellites (RAMS) techniques were cloned into the TA TOPO Cloning vector. Positive recombinant clones were then sequenced using the ABI PRISM 377 DNA sequencer.

Results and Discussion

The most widely distributed species in Peninsular Malaysia was *Vigna trinervia* with major concentrations in Bentong (Pahang), Bukit Kepong (Selangor), Pagoh (Johor) and Tangkak (Johor). Isoenzyme zones detected were alcohol dehydrogenase (ADH-1), aldolase (ALD-1), esterase (EST-1, EST-2 and EST-3), glutamate dehydogenase (GDH-1), malate dehydrogenase (MDH-1 and MDH-2), octanol dehydrogenase (ODH-1), glucose-6-phosphate dehydrogenase (G6PD-1) and general protein (GP-1, GP-2 and GP-3). Of these loci, five exhibited polymorphisms. They were EST-1, EST-2, EST-3, MDH-2 and GP-3. A maximum genetic distance of 0.025 was observed between Muar and Tangkak (Ladang Bekoh), while the least genetic distance of 0.003 was observed between populations in Tangkak (Town) and Bentong. Cluster analysis showed two distinct groups, with the first group for populations from Bentong, Tangkak (Ladang Bekoh) and Tangkak (Town) and the second group entirely for the population from Muar (Quah et al. and Ishak, 1998). In order to obtain greater information on the population structure in mungbean, DNA based markers such as Random Amplified Polymorphic DNA (RAPD) and microsatellites were used. Nine varieties of cultivated mungbean *Vigna radiata* and three wild populations of local *Vigna trinevia* (Bentong) were tested using RAPD markers. Five 10-*mer* primers, OPA-01, OPA-02, OPA-03, OPA-05 and OPA-07 revealed a total of 65 DNA markers of which a high percentage (95.38%) were polymorphic in the 12 populations. The wild varieties exhibited high genetic variability compared to the cultivated mungbean. The Bentong B13 wild samples revealed the highest genetic diversity within the population (81.7%) when compared to the other varieties. UPGMA cluster analysis of the 12 populations grouped all the nine varieties of cultivated mungbean into one major cluster while the other major cluster consisted of all three accessions of wild *Vigna* (Kumar *et al.*, 2000). Twelve microsatellite primer pairs from *Phaseolus*, a closely related species, were used to amplify the mungbean genome. Of the twelve, six were able to amplify microsatellite regions in *Vigna*. Four of these heterologous primer pairs (u18791, x04001, x13329 and x60000) amplified monomorphic loci while two (m75856 and x58274) amplified polymorphic sites when screened in domestic and wild mungbean populations (Kumar *et al.*, 1999). Due to the absence of DNA sequence data we have attempted to develop microsatellite primers specific for *Vigna*. Several strategies were developed which involves the use of Direct Amplification of Length Polymorphisms (DALP), RAPD and RAMS. The

DALP technique located many co-dominant regions but none was found to contain microsatellite repeats. The RAPD technique revealed two microsatellite loci while the RAMS technique was highly successful in that an estimated 80-microsatellite loci were identified. From these 51 primer pairs were designed. Twenty primer pairs were screened and 16 were found to be polymorphic.

Conclusions

The data collected formed a valuable database for comparison of genetic variation with related species and populations within the subgenus *Ceratotropis* and provide an insight into the genetic structure of populations and their evolutionary significance. Genetic markers are excellent in the characterisation of mungbean by helping in the selection of high variability populations for breeding programs in order to maximise heterosis. It also helps to test for correlations between markers and traits of economic importance. With an understanding of its genetic structure it will be possible to integrate marker-based technology into conventional breeding programs for mungbean and improve production of its cultivars.

Benefits from the study

The data collected formed a valuable database for comparison of genetic variation with related species and populations within the subgenus Ceratotropis and provide an insight into the genetic structure of populations and their evolutionary significance. Increased scientific knowledge as reported in scientific publications, which includes journal, conference and seminar papers as well as theses of undergraduate and postgraduate students.

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Project Publications in Refereed Journals

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Project Publications in Conference Proceeding

None.

Graduate Research

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