GENETIC STUDIES OF THE ASIAN SUBGENUS CERATOTROPIS

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Introduction

Ceratotropis is the most important subgenus under the genus Vigna and comprised of five major crops such as mungbean, black gram, adzuki bean, rice bean and moth bean. Its biodiversity includes wild species such as *V. trinervia, V. minima, V. reflexo-pilosa, V. radiata var sublobata, V. grandiflora,* and *V. umbellata var gracilis*. Genetic studies of Ceratotropis are important in that desirable genes from wild species could be incorporated into cultivated forms of the subgenus to enhance its commercial value. In Malaysia, preliminary collections of three wild species have been made by Bujang et al. (1994a; 1994b). A total of 183 accessions has been collected and evaluated using electrophoresis. Very little is known about the genetics of Asian Ceratotropis especially its breeding systems, cytogenetics, nodulating habits, general structure of populations and its phylogenetic relationships. In the present study, isozyme analysis and random amplified polymorphic DNA (RAPD) marker assay were used to evaluate both wild and cultivated species of Ceratotropis.

Materials and Methods

Isozyme analysis was used to evaluate collections of *Vigna trinervia* from four locations in Peninsular Malaysia while development of RAPD protocols were performed using cultivated varieties of *Vigna radiata*. In the case of isozymes, extracts of the seeds collected from Bentong, Muar and Tangkak (Ladang Bekoh and Tangkak) were subjected to vertical polyacrylamide gel electrophoresis at 4^0C and the gels were stained for the different enzymes. The data collected were analysed using the BIOSYS-1 computer package. A total of 15 varieties and varietal crosses of mungbean were used for the development of RAPD protocols. Genomic DNA was extracted from each variety using the Clontech Laboratories DNA extraction kit before RAPD protocols were developed. Twenty short arbitrary oligonucleotides (10-mer) with a 60-80% GC content from Operon Technologies (KitA) and ten arbitrary 16-24 mer oligonucletides with a GC content of 45-70% from Genosys Inc. were screened for PCR amplification and optimised to generate reproducible banding patterns.

Results and Discussion

Isozyme analysis revealed 13 loci for seven enzymes Alcohol dehydrogenase (ADH-1), Aldolase (ALD-1), Esterase (EST-1, EST-2 and EST-3), glutamate dehydrogenase (GDH-1), Malate dehydrogenase (MDH-1 and MDH-2), Octonol dehydrogenase (ODH-1), Glucose-6-phosphate dehydrogenase (G6PD-1) and general proteins (GP-1, GP-2 and GP-3) for *Vigna trinervia* accessions (Quah and Ishak,1998). Five of the 13 loci were polymorphic and were associated with EST, MDH and GP systems. The results showed a low level of genetic variation among the Vigna trinervia accessions. The highest genetic distance of 0.025 was observed between the populations from Muar and Tangkak (Ladang Bekoh) while the least distance of 0.003 was detected between populations from Tangkak (Town centre) and Bentong. The dendogram showed that *Vigna trinervia* could be positioned into two clusters, with one cluster for populations from Bentong, Tangkak (Ladang Bekoh) and Tangkak (Town centre) while the Muar population was confined to the second cluster. The optimised RAPD protocol using five short primers, OPA-01, OPA-04, OPA-06, OPA-15 and OPA-17 have been successfully applied to identify polymorphisms in the 15 varieties and varietal crosses (Kumar et al. 1998). The five primers managed to reveal genetic polymorphisms between and within different varieties and their crosses. However, more varieties and crosses will have to be studied before the genetic distances could be calculated and a phylogenetic tree drawn. The ten long arbitrary primers used were found to amplify mungbean DNA but the results were not as reproducible. Further optimisation need to be resolved before it could be used to differentiate mungbean accessions.

Conclusions

Both isozyme and RAPD markers are useful for genetic evaluation of mungbean. They could be used to complement other conventional approaches to improve breeding strategies in national breeding programmes.

References

Bujang, I., Egawa, Y., Tomooka, N., Tan, S.G., Abu Bakar, H.A., and Anthonysamy, S. 1994a. Exploration, Collection and Electrophoretic Variants of Wild Vigna (subgenus Ceratotropis) in Peninsular Malaysia. In: M.K. Vidyadaran and S.C. Quah (eds). Applied Biology Beyond the Year 2000. Proceedings of the 3rd Symposium of Malaysian Society of Applied Biology, Melaka. p. 51-55.

Bujang, I., Egawa, Y., Tomooka, N. and Quah, S.C. 1994b. Wild Vigna (subgenus Ceratotropis) species in Peninsular Malaysia. In: C.L. Koh (ed). The Role of genetics in National Development. Proceedings of The First National Congress on Genetics, Kuala Lumpur. p. 198-200.

Kumar, S.V., Quah, S.C. and Tan, S.G. 1998. Development of Random Amplified Polymorphic DNA Markers in mungbean (*Vigna radiata*). In: Mohamad Osman *et al* (eds). Genetics Towards Better Quality In Life. Proceedings of the Third National Congress on Genetics, UKM, Bangi. p. 239-241.

Quah, S.C and Ishak, M.Y. 1998. Isozyme variation in *Vigna trinervia* populations in Peninsular Malaysia. *Malaysian Applied Biology*. **27** (Suppl): 99-102.

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