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Variation of Cultivated Mungbean and Wild *Vigna* as Revealed by Random Amplified Polymorphic DNA Markers

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ABSTRAK

Variasi genetik sembilan varieti tamanan kacang hijau (Vigna radiata) *dan tiga populasi liar tempatan* Vigna (V. trinervia) *telah diselidik dalam kajian ini dengan menggunakan penanda RAPD. Sejumlah 65 fragmen DNA dengan saiz menjulat antara 173-1,500 bp telah dihasilkan daripada amplifikasi PCR menggunakan lima primer RAPD, di mana 95.38% fragmen adalah polimorfik. Analisis kelompok menunjukkan terdapat dua kumpulan utama, di mana kumpulan pertama terdiri daripada sembilan varieti* V. radiata *manakala kumpulan kedua terdiri daripada tiga populasi* V. trinervia. *Maklumat ini penting bagi pembiak baka tumbuhan agar dapat membuat keputusan yang tepat dalam usaha merangka program pembiakan atau kacukan bagi meningkatkan tanaman ini.*

ABSTRACT

The genetic variation of nine varieties of cultivated mungbean (Vigna radiata) *and three local populations of wild* Vigna (V. trinervia) *were evaluated in this study using RAPD markers. A total of 65 scorable DNA fragments ranging in size from 173–1,500 bp were obtained from the PCR amplification using five RAPD primers of which 95.38% were polymorphic. Cluster analysis revealed two major groups in which the first group consists of the nine varieties of* V. radiata, *while the second group includes the three populations of* V. trinervia. *This information is useful for plant breeders to make informed decisions in an effort to devise breeding or crossbreeding programmes for the development of the crop.*

INTRODUCTION

The Asian *Ceratotropis* is the most important subgenus under the genus *Vigna* and it comprises five major domesticated crops, the mungbean (*V. radiata* (L.) Wilczek), black gram (*V. mungo* (L.) Hepper), azuki bean (*V. angularis* (Willd.) Ohwi and Ohashi), rice bean (*V. umbellata* (Thunb.) Ohwi and Ohashi) and moth bean (*V. aconitifolia* (Jacq.) Maréchal (Baudoin and Maréchal 1988). Mungbean, in particular, is especially important as the major food crop under the subgenus *Ceratotropis* in developing countries in South and Southeast Asia where 80% of the world's mungbean are grown. It is rich in plant protein and is highly digestible, thus providing an alternative and inexpensive source of vegetable dietary protein. Since mungbean is a short duration legume (55 to 70 days) it is grown during the the inter-cropping season contributing to the farmers' income (Fernandez and Shanmugasundaram 1988).

However, mungbean is usually cultivated under low input conditions, thus improvement by breeding is important to increase yield. Breeding programs for mungbean improvements are specifically targeted at developing stable high yielding mungbean lines with resistance to disease and pest, uniform maturity, large seeded cultivars

and improved quality suitable for growth in the tropics and subtropics. As a result of breeding programs, hundreds of commercial varieties have been introduced with varying degrees of success (Shanmugasundaram 1988). To achieve varietal improvement effectively, it is important to incorporate wild stock into cultivated forms of mungbean to increase genetic variability in order to avoid any inbreeding depression and genetic bottlenecks in the future. Wild species generally exhibit a wide range of genetic diversity in terms of agronomic characteristics involving pest and disease resistance, maturity span, environmental adaptations and yield potential. Acknowledging the importance of wild plant stocks, several field surveys were conducted to collect wild samples of mungbean from various locations in Peninsular Malaysia, which resulted in *V. trinervia* being identified as the most dominant form of the local wild mungbean (Bujang *et al.* 1994).

Reports on the characterization of the mungbean genome using molecular markers are few. Recently Lakhanpaul *et al.* (2000) used Random Amplified Polymorphic DNA (RAPD) markers to characterize Indian mungbean varieties while Lambrides *et al.* (2000) used RAPD and Restriction Fragment Length Polymorphism (RFLP) markers to construct linkage maps for mungbean. RAPD markers have also been successfully used to characterize azuki bean (Yee *et al.* 1999; Xu *et al.* 2000) while linkage maps for crosses between the azuki bean and rice bean using RAPD and AFLP markers are now available (Kaga *et al.* 2000). In view of the success of using RAPD markers to characterize mungbean in other countries, we have used them to characterize the genetic relationships among several accessions of cultivated mungbean as well as local populations of wild *V. trinervia* from Peninsular Malaysia.

MATERIALS AND METHODS

Plant Material and Isolation of DNA

Seven varieties (V 1104, V 2273, V 2273-S, V 2773, V 3912, V 4717 and V 5973) and two varietal crosses (VC 3031A and VC 1131A) of domesticated *Vigna radiata* were obtained from the Asian Vegetable and Plant Research Center (AVRDC) while three populations of local wild mungbean *V. trinervia* designated as Bentong B13, Bentong B16 and 18 Bentong Std. respectively, were collected from Bentong district (Pahang state, Malaysia). Genomic DNA from seeds (30 seeds per variety/population) was extracted using a plant DNA extraction kit (Clontech Laboratories, USA). The extraction protocol followed that of the manufacturer's with minor modifications.

RAPD-PCR Amplification

Five arbitrary 10-mer primers OPA-01, OPA-02, OPA-3, OPA-05 and OPA-07 (Operon Technologies, USA), with 60-70% GC content, were used in this study (Table 1). The PCR reactions were carried out in a total volume of 10 µl containing 30 ng of genomic DNA, 2.5-4.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton-X 100, 0.5 mM each of dATP, dCTP, dGTP and dTTP, 10 pmol of primer and 2.5-5 U of *Taq* DNA polymerase (Promega, USA). PCR amplifications were performed in a PTC-150 Thermal Cycler (MJ Research Inc., USA) with the following temperatures: a predenaturation for 3 min at 96 °C, followed by 40 cycles of 10 s denaturation at 96 °C, 10 s annealing at 36 °C, 30 s extension at 72 °C and concluded with a 5 min final extension at 72 °C. The PCR products were separated according to size on a 2% agarose gel and visualized over ultraviolet light after ethidium bromide staining.

TABLE 1
Primers used in RAPD analysis and the magnesium chloride and *Taq* DNA polymerase concentrations for PCR amplification

Primer	Sequence 5' to 3'	Magnesium Chloride Concentration	*Taq* DNA Polymerase Concentration
OPA-1	CAGGCCCTTC	3.5 mM	2.5 U
OPA-2	TGCCGAGCTG	3.0 mM	2.5 U
OPA-3	AGTCAGCCAC	2.5 mM	2.5 U
OPA-5	AGGGGTCTTG	4.5 mM	5.0 U
OPA-7	GAAACGGGTG	4.0 mM	5.0 U

RAPD Data Analysis

Only reproducible RAPD markers were included in the analysis. The DNA bands produced were scored as 1 for presence and 0 for absence. The data matrix was then analyzed using the RAPDistance Package (Armstrong *et al.* 1994). Pairwise genetic similarity (S) was calculated using Nei and Li's (1979) similarity index. The unweighted pair group method with arithmetic averaging clustering (UPGMA; Sneath and Sokal 1973) was then performed based on genetic distance (D=1-S) using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC, Version 1.3) computer software program (Rohlf 1989).

RESULTS AND DISCUSSION

Identification of RAPD Bands

The five primers tested produced a total of 65 reproducible bands with a range in size of 173 to 1,500 bp. The number of amplified bands per primer varied from 10 to 18 (*Fig. 1*). The total number of polymorphic bands was 62 while the overall level of polymorphism calculated was 95.38% (Table 2). Only three bands, OPA1.7 (755 bp), OPA1.10 (445 bp) and OPA5.9 (390 bp) were monomorphic for all samples of the 12 accessions. There was however, one band, OPA3.4 (960 bp), which was shared exclusively by all the individual samples of *V. radiata* but was absent in all samples of *V. trinervia.* This band may prove useful as a diagnostic marker for differentiating between *V. radiata* and *V. trinervia.* It was found that variations within varieties were present but this was small and not reported here. However, the levels of variation within the wild *Vigna* populations were higher than the cultivated *Vigna.*

Genetic Distance and Cluster Analysis

Genetic distance between varieties ranged from 0.1425 to 0.4133. The highest genetic distance was observed between accessions Bentong B13 and V2273-S, while the lowest genetic distance was found between accessions VC 1131A and VC 3031A (Table 3). Cluster analysis based on UPGMA grouped the 12 accessions into two major clusters according to species. The first cluster consists of the nine varieties of *V. radiata* while the second cluster consists of the three populations of *V. trinervia* (*Fig. 2*).

In this study, the RAPD technique was found to be useful for identifying genetic variation in mungbean. The DNA fingerprinting pattern produced using five different RAPD primers enabled the twelve accessions to be distinguished from one another. The taxa studied show a high level of band sharing in the cultivated seeds. Any

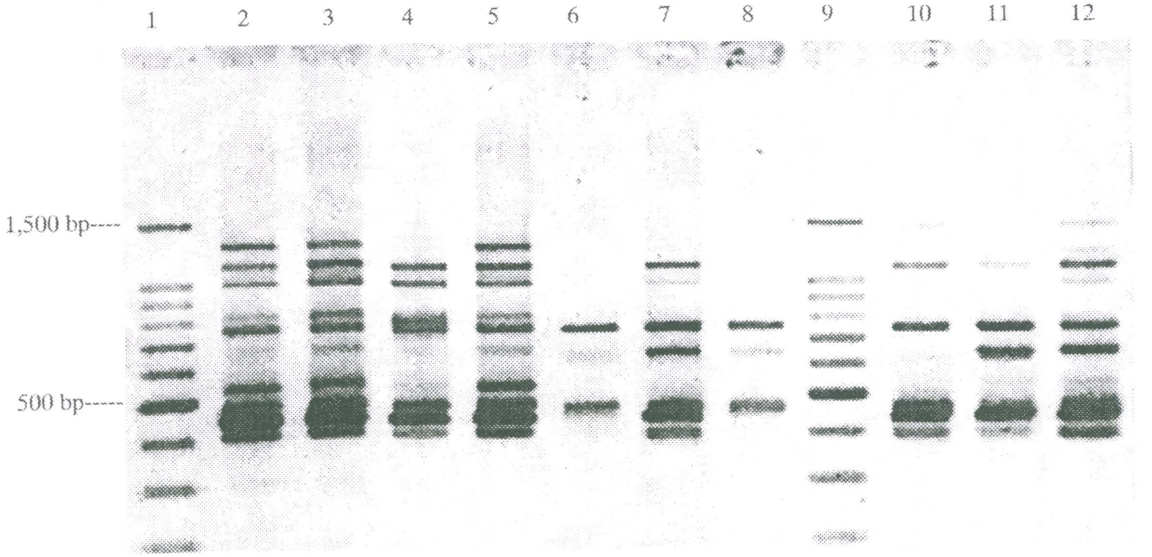

Fig. 1: PCR product generated by primer OPA-01 for the different varieties and populations. Lanes 1 and 9: 100 bp ladder. Lanes 2-5: Population 18 Bentong Std. Lanes 6-8: Varietal cross VC 1131A. Lane 10: Varietal cross VC3031A. Lanes 11 and 12: Variety V 3912

TABLE 2
RAPD profiles obtained from the five primers as observed in the *Vigna* accessions

	V2773	V2273-S	V2273	V1104	V3912	V4717	V5973	VC3031A	VC1131A	Bentong B13	Bentong B16	18 Bentong Std.
Number of Bands	52	53	51	56	51	54	55	56	49	51	51	52
Number of Polymorphic Bands	36	24	31	38	29	41	36	27	21	41	31	27
% of Polymorphic Bands	69.23	45.28	60.78	67.86	56.86	75.93	65.45	48.21	42.86	80.39	60.78	51.92
Total Number of Bands			65									
Total Number of Polymorphic Bands			62									
Total % Polymorphism			95.38									

TABLE 3
The pairwise genetic distance among the 12 accessions of *Vigna*

	V2773	V2273-S	V2273	V1104	V3912	V4717	V5973	VC3031A	VC1131A	Bentong B13	Bentong B16	18 Bentong Std.
V2773	—											
V2273-S	0.2132	—										
V2273	0.1923	0.1773	—									
V1104	0.2470	0.2163	0.2174	—								
V3912	0.2473	0.1854	0.2176	0.2365	—							
V4717	0.2741	0.2498	0.2474	0.2583	0.2561	—						
V5973	0.2398	0.1916	0.1986	0.2197	0.2069	0.2219	—					
VC3031A	0.2025	0.1586	0.1855	0.2130	0.1983	0.2440	0.1714	—				
VC1131A	0.2137	0.1617	0.1926	0.2029	0.1885	0.2522	0.1868	0.1425	—			
Bentong 13	0.3633	0.4133	0.3796	0.3811	0.3744	0.3950	0.3770	0.3645	0.3695	—		
Bentong B16	0.3392	0.4005	0.3632	0.3639	0.3538	0.3760	0.3699	0.3447	0.3551	0.2005	—	
18 Bentong Std.	0.3291	0.3792	0.3552	0.3539	0.3335	0.3622	0.3497	0.3269	0.3256	0.1914	0.1488	—

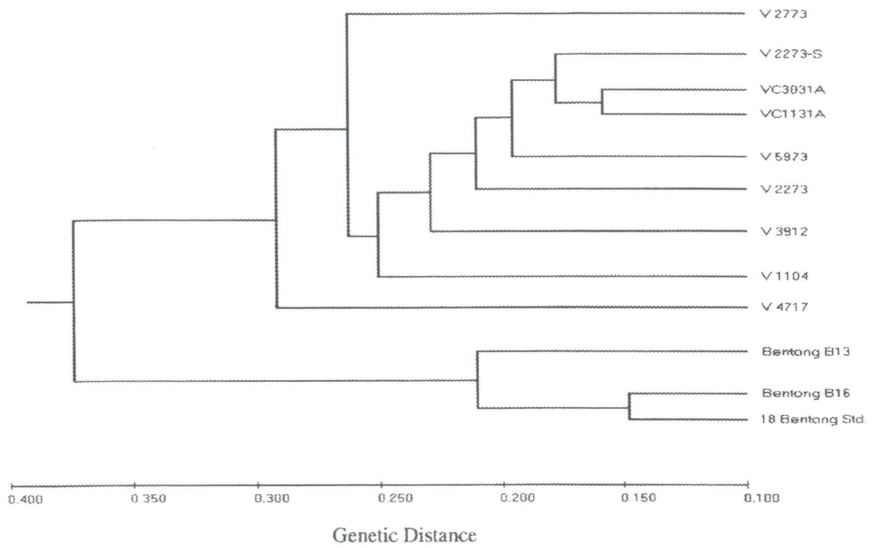

Fig. 2: UPGMA dendrogram showing the relationship among the 12 Vigna *accessions*

pair of individuals examined shared more than 70% of all the bands. Overall, the study showed that a high percentage of the RAPD markers were polymorphic when compared among the accessions. Based on the genetic distance values, the wild populations exhibited higher genetic distances compared to the domesticated varieties. This indicates a larger gene pool and higher levels of diversity in the wild populations. The Bentong B13 in particular, reveals higher genetic diversity compared to the other two populations from Bentong. Although the presence of a diagnostic marker that was able to differentiate between *V. radiata* and *V. trinervia* was identified, screening populations of *V. trinervia* from other geographical areas are needed to confirm this finding. Diagnostic markers will be useful since it is difficult to differentiate between the two species in the absence of seeds and flowers.

The RAPD-based dendrogram obtained from the cluster analysis is important because it reveals the relationships among the different accessions. It is expected that crosses between varieties that are genetically distant would demonstrate maximum heterosis or hybrid vigour. Despite the fact that there are numerous accessions of cultivated mungbean available commercially, the success rate of breeding programs has been poor. This may be partly due to the fact that different environmental conditions can affect the potential exploitation of the bean. To overcome this problem, local wild accessions are often selected and crossed with potential cultivar lines to confer some protection against pests.

A lot of focus has been given to *V. radiata* var. *sublobata* as a gene source for mungbean improvement since it is suspected to be the wild ancestor and putative progenitor of mungbean (Lukoki *et al.* 1980). The wild species are especially important since desirable genes from it could be incorporated into cultivated forms to enhance its economic value (Tomooka *et al.* 1992a). For example, *V. r. sublobata* showed complete resistance to the azuki weevil (Fujii and Miyazaki 1987). It also exhibits tolerance to Yellow Mosaic Virus, has high methionine content in the seeds, high photosynthetic efficiency and drought tolerance (Singh and Ahuja 1977; Babu *et al.* 1988). By using *V. r. sublobata* as a gene source, Tomooka *et al.* (1992a) successfully developed a bruchid resistant mungbean line in Thailand.

V. trinervia was initially classified and treated as *V. r. sublobata* until 1985 when it was recognized as a distinct species (Tateishi 1985). Crosses between *V. r. sublobata* and *V. trinervia* were successfully developed by Egawa *et al.* (1996). *V. r. sublobata* can also be easily crossed with *V. radiata* and no genetic barrier has been found (Dana 1966). Crosses between *V. radiata* and *V. trinervia* can be carried out and this may confer the same bruchid resistance to domesticated varieties. Other wild *Vigna* such as *V. minima* and *V. reflexo-pilosa,* which are also found in Malaysia,

may confer similar if not other potentially economic or commercially important traits (Tomooka *et al.* 1992b). Wild populations often contain genes, which confer resistance to diseases and pests and are more adapted to environmental stress when compared to modern cultivated species (Harlan 1976).

Although RAPD markers have been frequently applied in population genetic analysis and in mapping studies, their use has been limited because of frequent reports of extreme sensitivity to amplification conditions and poor reproducibility. In spite of this, RAPD analysis is favored over other genetic markers such as RFLP or microsatellite analysis because of the ease with which genetic information is identified without prior sequence knowledge. Nevertheless, if reproducibility of the markers is assured, the RAPD markers could then be further exploited to identify loci controlling disease resistance and traits of economic importance. This knowledge is imperative in order to improve breeding strategies in mungbean breeding programs.

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