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# SPECIES DIVERSITY AND RELATIONSHIP AMONG *VIGNA RADIATA* (L.) WILCZEK AND ITS CLOSE WILD RELATIVES

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*Key words:* Species diversity, Microsatellite marker, STMS, Relationship

**Abstract**

The genetic variation population substructure and phylogenetic relationships between wild forms and cultigens of *Vigna,* statistics was calculated for all 10 loci for 390 individuals of 26 populations belonging to five *Vigna* species. Twenty six accessions were categorized in four groups. The percent of polymorphic loci varied from 10 to 50. The estimates for observed and effective number of alleles per locus ranged from 1.10 to 1.90 and 1.01 to 1.53, respectively. The Nei's genetic diversity estimates ranged from 0.04 to 0.25. The values for expected heterozygosity ranged from 0.01 to 0.26. The Shannon-Weaver information index values varied from 0.04 to 0.41. Inter-species variability was analyzed by computing the parameters, number of polymorphic loci, percent polymorphic loci, observed number of alleles per locus, effective number of alleles per locus, Shannon-Weaver information index, observed heterozygosity, expected heterozygosity, F-statistics and gene flow. Dendrogram based on STMS profiles indicated that *V. radiata*, *V. radiata* var. *sublobata* and *V. radiata* var. *setulosa* are distinct taxonomic groups. Considerable gene-flow was detected between their populations.

## Introduction

Mungbean, *Vigna radiata* (L.) Wilczek is an important grain legume crop in Asian agriculture. Almost 90% of world's mungbean production is produced in Asia and India is the world's largest producer accounting for more than 50% of world's production (Vijayalakshmi *et al.* 2003). *Vigna radiata* var. *sublobata* belongs to the subgenus *Ceratotropis* is considered to be a wild ancestor of mungbean (Marechal *et al.* 1978). *Vigna radiata* var. *setulosa* and *Vigna hainiana* are very closely related to *Vigna radiata* (Pandiyan *et al.* 2010).

Several studies using molecular marker have contributed to our understanding of the estimation of genetic diversity and relationship between accessions and species and map-based cloning of agriculturally interesting genes (Winter and Kahl 1995). Fatokun *et al.* (1993) used RFLP analysis of nuclear sequence to study the genetic relationships in 18 species belonging to four subgenera of the genus *Vigna*. The genetic variation among five species in the subgenus *Ceratotropis* was investigated by Kaga *et al.* (1996) using RAPD and was able to divide this subgenus into two major subgroups. Sonnante *et al.* (1996) used isozyme variation to study the relationship between *V. unguiculata* and other species of section *Vigna*. Finally, Vaillancourt and Weeden (1996) investigated the position of *V. unguiculata* in the genus using both chloroplast DNA and isozyme polymorphisms. Probably the best markers in this respect descend from small, tandemly arranged repetitive elements called microsatellites, simple-sequence repeats (SSRs) or simple tandem repeats (STRs). Mutation rates of SSRs are high: 2.5 × 10 - 5 to 1 × 10 - 2 mutations per locus per gamete per generation have been estimated (Weber and Wong 1993), which accounts for their polymorphism. Litt and Luty (1989) converted SSRs in markers and named sequence-tagged microsatellite marker (STMSs). These STMSs are co-dominant markers

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like RFLPs. However, the number of alleles they detect in a population is usually much higher as compared to RFLPs. Present investigation was focused on the genetic relationships of mungbean and other three *Ceratotropis* species and comparison was also made between cultivated and wild forms.

## Materials and Methods

A total of 26 accessions of *Vigna* were studied (Table 1). Fifteen seeds per accession were set in a single row on wet paper towel and rolled wet paper towel were placed in BOD at 30 ± 2°C in absence of light. Total genomic DNA was extracted from 15 days old individual etiolated seedling using CTAB extraction procedure (Doyle and Doyle 1990). The quality and concentration of extracted DNA were estimated by using a UV-Vis spectrophotometer at 260/280 nm using different concentrations of phage λ DNA (Toyobo Co. Ltd., Japan) as standards. A diluted stock of 20 ng/µl DNA was used for setting up PCR amplification reactions.

**Table 1. List of *Vigna* accessions used for STMS analysis.**

| Sl. No. | Species | Accession | Cultvar/wild |
|---|---|---|---|
| 1 | *V. radiate* | IC- 413825 | Landrace |
| 2 | | PDM-54 | " |
| 3 | | PDM-11 | " |
| 4 | | SAMRAT | " |
| 5 | | ML-131 | " |
| 6 | | SML-32 | " |
| 7 | | PDM-139 | " |
| 8 | | IC-251431( TCR-79) | " |
| 9 | | IC-251429( TCR- 77) | " |
| 10 | | IC-251424( TCR-72) | " |
| 11 | *V. rediata* var. *sublobata* | IC- 277010 | Wild |
| 12 | | IC- 277058 | " |
| 13 | | IC- 277038 | " |
| 14 | | IC- 277024 | " |
| 15 | | IC-277019 | " |
| 16 | | IC-276990 | " |
| 17 | | BB-14-O1A | " |
| 18 | | KPS-1-KALLAGHAT | " |
| 19 | | CHITTORGARH | " |
| 20 | | KPS-DAOLLAGHAT | " |
| 21 | *V. hainiana* | BBD-5-01B | " |
| 22 | | BB-21-01A | " |
| 23 | | BBD-15-01B | " |
| 24 | | BB 2623 | " |
| 25 | | IC251381(TCR 29) | Land race |
| 26 | *V. setulosa* | BBD-9-01B | Wild |

Thirty primer pairs were screened for repeatability and scorability using two samples each of *V. radiata* and *V. hainiana.* Out of these 30 primers, 10 primers yielding repeatable, good and polymorphic amplification products were selected. The nucleotide sequences of these primers and characteristics of amplification products recorded are listed in Table 2. The PCR reactions were

carried out in a DNA thermal cycler (Gene Amp 9600 PCR system, Perkin Elmer Cetus, Norwark, CT ). Each 25 µl reaction mixture contained 1X reaction buffer (10 mM Tris pH 8.3 and 50 mM KCl), 3 mM $MgCl_2$, 1U of Taq DNA polymerase, 200 µM each of dNTPs, 0.6 µM of primer and approximately 30 ng of template DNA. The PCR amplification conditions were as follows: initial extended step of denaturation at 94°C for 3 min followed by 30 cycles of denaturation (94°C for 1 min), primer annealing (for 1 min, temperature specified in Table 2) and primer elongation (72°C for 1 min), followed by extended elongation step at 72°C for 5 min. Reaction products were mixed with 2.5 µl of 10X loading dye and spun briefly in a microfuge before loading (Sambrook *et al.* 1989). The amplification products were electrophoresed on 6% PAGE at 100 volts. Gels were stained with ethidium bromide and photographed on Polaroid 667 film under ultra violet light of 250 nm weavelength.

**Table 2. Primer sequences repeat unit and annealing temperature for STMS analysis.**

| Sl. No. | Primer | Repeat unit | Annealing | Sl. No. | Primer | Repeat unit | Annealing |
|---|---|---|---|---|---|---|---|
| 1 | VM 21 | (AT) | 48 | 6 | MB 122A | (TGGT) | 48 |
| 2 | VM 22 | (AG) | 48 | 7 | AB 128079 | (AG) | 55 |
| 3 | VM 24 | (AG) | 55 | 8 | AB 128093 | (AG) | 55 |
| 4 | VM 27 | (AAT) (TC) (AC) | 48 | 9 | AB 128113 | (AG) | 55 |
| 5 | VM 31 | (CT) | 55 | 10 | AB 128135 | (AG) | 55 |

The STMS genotype data matrixces were used for assessing genetic diversity and structure in a hierarchical manner from overall accessions. The among type genetic diversity was calculated by considering all genotyped individuals of a given type as one population while genetic parameters for among accessions was calculated based on 15 individuals per accession. Genetic similarity between each pair of species was estimated using the method of Jaccard (1908): $GS = n_{xy}/n_t - n_z$, where $n_{xy}$ is the number of bands common to accession A and B; nt the total number of bands present in all samples and nz the number of bands not present in both A and B but found in other samples. The amount of genetic variation within each population and species was quantified by determining number of polymorphic loci, per cent polymorphic loci (p), observed number of alleles per locus (na) and effective number of alleles per locus (ne) (Nei 1987). Expected heterozygosity was calculated for all populations using the procedure of Lynch and Milligan (1994). FST values representing differentiation between populations were also calculated using Arlequin Version 3.01 software (Excoffier *et al.* 1992). The Shannon-Weaver information index (Shannon and Weaver 1949) was calculated to measure the extent of diversity in each sample. The Wright's F-statistics, FCT, FSC and FST were also computed (Wright 1965). These are hierarchically related descriptors of the distribution of genetic variation within and among populations. The statistical analyses were performed using POPGEN version 1.31. An analysis of molecular variance (AMOVA) was also performed to partition the total genetic variation into that occurring within population, among population within groups and among groups using Arlequin Version 3.01 software (Excoffier *et al.* 1992). The dendrogram was constructed using UPGMA procedure to study the differences among the populations within a cluster.

## Results and Discussion

Genetic variation statistics was calculated for all 10 loci for 390 individuals of 26 populations belonging to five *Vigna* species. Twenty six accessions were categorized in four groups. The number of alleles detected from 1 to 5 with the average number of 2.8 alleles per primer pair and a

standard deviation of 1.32. The pair-wise similarity values between species of radiata- radiate complex ranged from 0.021 to 0.931 with the average value of 0.356. The FST values between populations ranged from 0.001 (between accessions of *V. hainiana* and *V. radiata* var. *sublobata* i.e. BBD-5-01B and IC-277038) to 0.911 (between accessions of *V. radiata* and *V. radiata* var. *setulosa* i.e. BBD-9-01B and IC-251431). The wild *Vigna* forms showed higher intraspecific variability in comparison to the cultivars (Tables 3, 4). Diversity parameters in the populations were indicators of the diversity prevalent in each of the species analyzed (Table 4). The number of polymorphic loci ranged from 1 to 5 and the per cent of polymorphic loci varied from 10 to 50%. The estimates for observed and effective number of alleles per locus ranged from 1.10 to 1.90 and 1.01 to 1.53, respectively. The Nei's genetic diversity (h) estimates ranged from 0.04 to 0.25. The values for expected heterozygosity ranged from 0.01 to 0.26. The Shannon-Weaver information index values varied from 0.04 to 0.41.

**Table 3. Genic diversity parameters for the *Vigna* population based on STMS polymorphism.**

| Population code | Sample size | No. of poly-morphic loci | % poly-mor-phic loci (P) | Observed number of alleles per locus (na) | Effective number of alleles per locus (ne) | Nei's genic diversity (h) | Shannon-Weaver information index (I) | Exp. heterozy-gosity |
|---|---|---|---|---|---|---|---|---|
| BBL9 | 30 | 5 | 50 | 1.90 ± 1.10 | 1.53 ± 0.62 | 0.25 ± 0.27 | 0.41± 0.46 | 0.26 ± 0.28 |
| BBL38 | 30 | 5 | 50 | 1.70 ± 0.82 | 1.37 ± 0.51 | 0.25 ± 0.23 | 0.32 ± 0.37 | 0.20 ± 0.28 |
| BBL43 | 30 | 3 | 30 | 1.40 ± 0.69 | 1.13 ± 0.25 | 0.09 ± 0.15 | 0.15 ± 0.26 | 0.09 ± 0.16 |
| BBL57 | 30 | 2 | 20 | 1.20 ± 0.42 | 1.11 ± 0.26 | 0.07 ± 0.15 | 0.10 ± 0.22 | 0.07 ± 0.15 |
| BB21 | 30 | 1 | 10 | 1.10 ± 0.31 | 1.08 ± 0.25 | 0.04 ± 0.14 | 0.06 ± 0.20 | 0.04 ± 0.14 |
| BBD09 | 30 | 2 | 20 | 1.20 ± 0.42 | 1.10 ± 0.31 | 0.06 ± 0.16 | 0.08 ± 0.21 | 0.06 ± 0.16 |
| BBD05 | 20 | 4 | 40 | 1.70 ± 0.94 | 1.46 ± 0.66 | 0.20 ± 0.27 | 0.33 ± 0.45 | 0.22 ± 0.29 |
| BBD15 | 18 | 5 | 50 | 1.60 ± 0.70 | 1.38 ± 0.59 | 0.19 ± 0.27 | 0.30 ± 0.37 | 0.20 ± 0.25 |
| BBL29 | 14 | 5 | 50 | 1.50 ± 0.52 | 1.36 ± 0.42 | 0.20 ± 0.22 | 0.30 ± 0.32 | 0.22 ± 0.24 |
| BBL77 | 30 | 2 | 20 | 1.30 ± 0.67 | 1.23 ± 0.52 | 0.10 ± 0.22 | 0.16 ± 0.35 | 0.11 ± 0.23 |
| BB03 | 28 | 1 | 10 | 1.10 ± 0.31 | 1.01 ± 0.04 | 0.01 ± 0.04 | 0.02 ± 0.08 | 0.01 ± 0.04 |
| BB14 | 30 | 1 | 10 | 1.10 ± 0.31 | 1.08 ± 0.25 | 0.04 ± 0.14 | 0.06 ± 0.20 | 0.04 ± 0.14 |
| SAMRAT | 30 | 1 | 10 | 1.10 ± 0.31 | 1.08 ± 0.25 | 0.04 ± 0.14 | 0.06 ± 0.20 | 0.04 ± 0.14 |
| PDM54 | 30 | 1 | 10 | 1.10 ± 0.31 | 1.03 ± 0.09 | 0.02 ± 0.07 | 0.04 ± 0.12 | 0.02 ± 0.07 |
| PDM11 | 30 | 2 | 20 | 1.20 ± 0.42 | 1.16 ± 0.35 | 0.09 ± 0.19 | 0.12 ± 0.27 | 0.09 ± 0.19 |
| ML131 | 30 | 3 | 30 | 1.30 ± 0.48 | 1.25 ± 0.42 | 0.14 ± 0.22 | 0.19 ± 0.31 | 0.14 ± 0.23 |
| SML32 | 30 | 3 | 30 | 1.40 ± 0.69 | 1.32 ± 0.60 | 0.15 ± 0.24 | 0.22 ± 0.38 | 0.15 ± 0.25 |
| PDM139 | 30 | 1 | 10 | 1.10 ± 0.31 | 1.10 ± 0.31 | 0.05 ± 0.15 | 0.06 ± 0.21 | 0.05 ± 0.16 |
| IC251424 | 30 | 1 | 10 | 1.10 ± 0.31 | 1.10 ± 0.04 | 0.01 ± 0.04 | 0.02 ± 0.07 | 0.01 ± 0.04 |
| Chittor | 30 | 3 | 30 | 1.50 ± 0.84 | 1.32 ± 0.58 | 0.15 ± 0.24 | 0.24 ± 0.40 | 0.15 ± 0.25 |
| IC251431 | 30 | 4 | 40 | 1.40 ± 0.51 | 1.37 ± 0.48 | 0.19 ± 0.25 | 0.27 ± 0.34 | 0.20 ± 0.25 |
| BB2623 | 30 | 5 | 50 | 1.70 ± 0.82 | 1.36 ± 0.61 | 0.17 ± 0.24 | 0.28 ± 0.40 | 0.17 ± 0.25 |
| IC251429 | 30 | 1 | 10 | 1.10 ± 0.31 | 1.08 ± 0.25 | 0.04 ± 0.14 | 0.06 ± 0.20 | 0.04 ± 0.14 |
| KPS Kalla | 30 | 1 | 10 | 1.10 ± 0.31 | 1.10 ± 0.31 | 0.05 ± 0.16 | 0.07 ± 0.21 | 0.05 ± 0.16 |
| IC251381 | 30 | 2 | 20 | 1.20 ± 0.42 | 1.20 ± 0.41 | 0.10 ± 0.21 | 0.14 ± 0.29 | 0.10 ± 0.21 |
| KPS Dao11 | 30 | 1 | 10 | 1.10 ± 0.31 | 1.08 ± 0.25 | 0.04 ± 0.14 | 0.06 ± 0.20 | 0.04 ± 0.14 |

Inter-species variability was analyzed by computing the parameters, number of polymorphic loci (P), per cent polymorphic loci, observed number of alleles per locus (na), effective number of alleles per locus (ne), Shannon-Weaver information index (I), observed heterozygosity (Ho),

expected heterozygosity (He), F-Statistics (Fis, Fit, Fst) and gene flow (Nm) (Table 4). Maximum number of polymorphic loci (8) and per cent polymorphic loci (80) were observed for *V. hainiana* while, minimum number of polymorphic loci (2) and per cent polymorphic loci (20) were observed for *V. radiata* var. *setulosa*. Observed number of alleles per locus (na) and effective number of alleles per locus (ne) ranged from 2.50 (*V. radiata* var. sublobata) to 1.20 (*V. radiate* var. *setulosa*) and 1.83 (*V. hainiana*) to 1.11 (*V. radiata* var. *setulosa*), respectively. Shannon-Weaver information index (I) ranged from 0.08 (*V. radiata* var. *setulosa*) to 0.58 (*V. hainiana*). The Nei's genetic diversity (h) estimates ranged from 0.06 (*V. radiata* var. *setulosa*) to 0.34 (*V. hainiana*). The gene-flow among populations within species varied from 0 to 0.18 (*V. hainiana*). Wright fixation indices (FSC, FST and FCT) were calculated to evaluate population subdivision and population substructure. The computations for these indices were performed for all the populations. The average F- statistics over all loci was calculated to be FSC (0.71), FST (0.76), and FCT (0.18). Considerable gene-flow was detected between the populations of *V. radiata*, *V. radiata* var. *sublobata* and *V. hainiana.* This indicates occurrence of intercrossing between wild populations of these species, as these populations occur in contiguous areas and mostly their geographic areas of occurrence overlap.

**Table 4. Assessment of genetic divergence statistics among four *Vigna* species based on STMS markers.**

| Group | Sample size | No. of poly-morphic loci (P) | % poly-morphic loci | Observed number of alleles/locus (na) | Effective number of alleles/locus (ne) | Shannon-Weaver information index (I) |
|---|---|---|---|---|---|---|
| *V. radiate* var. *sublobata* | 284 | 7 | 70 | 2.50 ± 1.26 | 1.67 ± 0.63 | 0.54 ± .47 |
| *V. radiata* | 298 | 5 | 50 | 1.70 ± 0.82 | 1.33 ± 0.46 | 0.30 ± 0.35 |
| *V. hainiana* | 128 | 8 | 80 | 2.40 ± 1.07 | 1.83 ± 0.88 | 0.58 ± 0.48 |
| *V. setulosa* | 30 | 2 | 20 | 1.20 ± 0.42 | 1.11 ± 0.31 | 0.084 ± 0.22 |

(Right side of the table)

| Observed heterozygosity (Ho) | Expected heterozygosity (He) | Nei | Fis | Fit | Fst | Gene flow (Nm) |
|---|---|---|---|---|---|---|
| 0.024 | 0.31 ± .27 | 0.31 ± 0.27 | 0.7502 | 0.912 | 0.648 | 0.14 |
| 0.817 | 0.18 ± 0.21 | 0.18 ± 0.22 | 0.4042 | 0.783 | 0.636 | 0.15 |
| 0.657 | 0.34 ± 0.28 | 0.34 ± 0.27 | 0.6991 | 0.874 | 0.582 | 0.18 |
| 0.942 | 0.06 ± 0.16 | 0.06 ± 0.16 | - | –0.890 | 0.000 | - |

AMOVA (Table 5) indicated that 23.55% of the total variation was accounted by within population variation in comparison to 58.09% by among population variation within the species, and among species variation accounted for 18.36% of total variation. The segregating of molecular variance with AMOVA revealed that extensive variation exists among the species and populations indicating that greater number of population samples should be sampled and studied to delineate the species identities and boundaries in this species complex. The UPGMA tree was constructed on the basis of Jaccard's similarity matrix for all 26 species. Dendogram comprising (Fig. 1) showed that it was divided into two main clusters. Cluster I showed that *V. hainiana* (BB2623) was closely related and was in the same cluster with the three accession of *V. sublobata.* Cluster II was divided further into many subclusters. Distinctively the accessions of *V. radiata* (Samrat, IC251429, and PDM 54) were almost alike. The wild crop *V. setulosa* was although in one sub

cluster but was diverse from rest of the accessions. In the same sub cluster wild relative *V. hainiana* was mixed with the cultivated crop *V. sublobata.* Similarly in one sub cluster one accession of *V. sublobata* was grouped with the accessions of *V. radiata.* UPGMA tree based on STMS profiles indicated that *V. radiata*, *V. radiata* var. *sublobata* and *V. radiata* var. *setulosa* are

**Table 5. Analysis of molecular variance (AMOVA) among the groups and within population.**

| Source of Variation | df | Sum of squares | Variance component | Percentage variation |
|---|---|---|---|---|
| Among groups | 3 | 187.636 | 0.48752 Va | 18.36 |
| Among population within groups | 22 | 495.281 | 1.54278 Vb | 58.09 |
| With populations | 344 | 215.116 | 0.62534 Vc | 23.55 |
| **Total** | **369** | **898.032** | **2.65565** | |

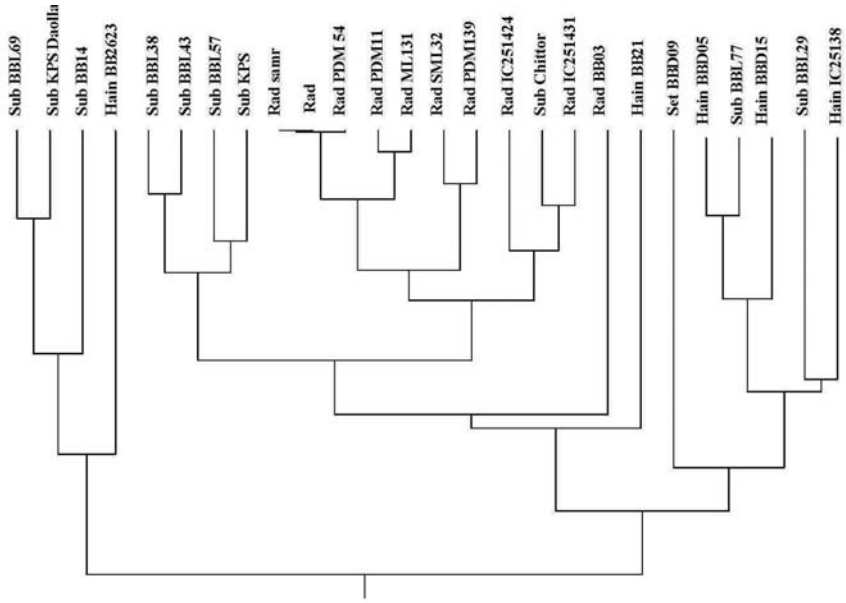


Fig. 1. Genetic distance dendrogram depicting cluster formation of all 26 accessions of *Vigna* species. The dendrogram was generated from selected data on 10 primers. Dendrogram based Nei's (1972) genetic distance: Method = UPGMA.

distinct taxonomic groups. However, as these species were placed in the same cluster in the dendrogram, their origin from a common ancestor is evident. In addition, cultivated forms are grouped close to their proposed wild forms. However, elaborate analysis of diverse populations is required to further confirm whether *V. hainiana* could be considered as the putative progenitor of green gram and black gram and whether the closest wild relative of green gram, namely *V. radiata* var. *sublobata* and *V. radiata* var. *setulosa*. To conclude, the evidences suggest that the wild species, *V. radiata* var. *sublobata* has high similarity to its respective cultivated forms and *V. hainiana* could be the pivotal progenitor species of *V. radiata* var. *sublobata* from which the cultivated mung (*V. radiata*) have evolved.

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**References**

Doyle JJ and Doyle JL 1990. Isolation of plant DNA from fresh tissues. Focus **12**: 13-15.

Excoffier L, Smouse PE and Quattro JM 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Applications to human mitochondrial DNA restriction sites. Genetics **131**: 479-491.

Fatokun CA, Danesh D, Young ND and Stewart E 1993. Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. Theor. Appl. Genet. **86**: 97-104.

Jaccard P 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. **44**: 223-270.

Kaga A, Tomooka N, Egawa Y, Hosaka K and Kamijima O 1996. Species relationships in subgenus *Ceratotropis* (genus *Vigna*) as revealed by RAPD analysis. Euphytica **88**: 17-24.

Litt M, Luty JA 1989. A hypervariable microsatellite revealed by i*n vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. Amer. J. Hum. Genet. **44**: 397-401.

Lynch M and Milligan BG 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. **3**: 91-99.

Marechal R, Mascherpa JM and Stainer F 1978. Etude taxonorniqued'un group complexe d'especes des genres *Phaseolus* et *Vigna* (Papillionaceae) sur la base de donnees morphologiques, et polliniques traitees par l'analyse informatique. Boissiera **28**: 1-273.

Nei M 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Pandiyan M, Senthil N, Ramamoorthi N, Muthiah AR, Tomooka N, Duncan V and Jayaraj T 2010. Interspecific hybridization of *Vigna radiata* × 13 wild *Vigna* species for developing MYMV donar. Electron. J. Plant Breed. **1**(4): 600-610.

Sambrook, J, Fritsch EF and Maniatis T 1989. Molecular cloning. New York: Cold Spring Harbor Laboratory Press. **2**: 14-9.

Shannon CE and Weaver W 1949. The mathematical theory of communication. University of Illinois Press, Urbana.

Sonnante G, Piergiovanni AR, Ng QN and Perrino P 1996. Relationships of (L.) Walp., *V. vexillata* (L.) A. Rich. and species of section *Vigna* based on isozyme variation. Genet. Res. and Crop Evol. **43**: 157-165.

Vaillancourt RE and Weeden NF 1996. *Vigna unguiculata* and its position within the genus *Vigna*. *In:* Advances in Legume systematic 8: Legumes of Economic Importance, B. Pickersgill and J.M. Lock (eds). pp. 89-93. Royal Botanic Gardens, Kew, London.

Verdcourt B 1970. Studies in the Leguminosae-Papilionoideae for the Flora of Tropical East Africa. IV. Kew Bull. **24**: 507-569.

Vijayalakshmi P, Amirthaveni S, Devadas RP, Weinberger K, Tsou SCS and Shanmugasundaram S 2003. Enhanced bioavailability of iron from mungbeans and its effects on health of school children. AVRDC-World Vegetable Center.

Weber JL and Wong C 1993. Mutation of human short tandem repeats. Hum. Mol. Genet. **2**: 1123-1128

Winter P and Kahl G 1995. Molecular marker technologies for plant improvement. World J. Microbiol. Biotechnol. **11**: 438-448.

Wright S 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution **19**: 395-420.

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