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Study of Phylogenetic Relationship among *Vigna* Species Using Morphological Characters and Seed Storage Proteins

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Abstract: The experiment was conducted to study the phylogenetic relationship among four cultivated *Vigna* species, including *Vigna radiata*, *Vigna mungo*, *Vigna aconitifolia* and *Vigna unguiculata*, using the germplasm from National Genebank of Pakistan. Seed of 15 accessions of each species was taken from Genebank of Plant Genetic Resources Institute, NARC, Islamabad, and these 60 accessions were planted in the NARC field, using augmented design. Data was recorded for eight morphological characters, including branching pattern, calyx color, corolla color, flowering period, leaf senescence, petiole color, petiole length and twining tendency. Cluster analysis was conducted, using software Statistica through Ward's method. The seed of all 60 accessions was subjected to SDS-PAGE analysis. Cluster analysis of seed storage proteins was conducted using Unweighted Pair-group Average method. Cluster analysis for morphological characters divided the accessions into four clusters of different species. In case of seed storage proteins, 13 clusters were observed, five of which included accessions of different species. It was found by cluster analysis for morphological characters that *Vigna radiata* and *Vigna mungo* are in same major cluster at linkage and they are related to *Vigna unguiculata* at greater linkage distance. Phylogenetic tree constructed on the basis of morphological characters indicates genetic relationship among cultivated *Vigna* species. Seed storage protein analysis gives an estimate of diversity in protein profile and the possible genetic relationship.

Key words: *Vigna*, Morphology, SDS-PAGE, Genetic diversity, Phylogeny.

INTRODUCTION

Genus *Vigna* consists of more than 200 species including some cultivated species that are of tremendous agronomic importance. The cultivated *Vigna* species are grown as pulses mainly in the warm temperate and tropical regions. These are important due to high and easily di**g**estible proteins in grains. These crops are also used as forage, green manure, and cover crops. These crops are suitable as catch crops and can fit well in intercropping, mixed or relay cropping due to their short life cycle (Pratap *et al*., 2013). Mung bean (*Vigna radiata*), mash bean (*Vigna mungo*), moth bean (*Vigna aconitifolia*) and cowpea (*Vigna unguiculata*) are among the most important pulses in the world. Mung bean and mash bean are included in the major pulses crops in Pakistan. During 2014, mung bean, also known as green gram, was grown in Pakistan, over an area of 130900 hectares with a production of 92900 tonnes, while mash bean, also known as blackgram, was cultivated on 20900 hectares with a production of 10400 tonnes (Government of Pakistan, 2015a). Moth bean and cowpea are cultivated as minor pulses. During 2013-2014, Pakistan imported 499300 tonnes of pulses with a value of 31526.2 million Rupees (Government of Pakistan, 2015b). There is a need to improve the production of pulses in Pakistan, to cater for the increasing demand.

Genebank of Plant Genetic Resources Institute, National Agricultural Research Center (NARC), Islamabad, has germplasm collection of *Vigna* species. There is a need to study the extent of variation in the germplasm for its utilization in crop improvement. Phylogenetic relationship among related species is important for plant breeders to transfer economically important traits (Douglas and Soltis, 2003). The present experiment was conducted to study the phylogenetic relationship among mung

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bean (*Vigna radiata*), mash bean (*Vigna mungo*), moth bean (*Vigna aconitifolia*) and cowpea (*Vigna unguiculata*) germplasm accessions for morphological traits and seed storage proteins.

MATERIALS AND METHODS

Four species of *Vigna* including mung bean (*Vigna radiata*), mash bean (*Vigna mungo*), moth bean (*Vigna aconitifolia*) and cowpea (*Vigna unguiculata*) were studied for morphological and biochemical analysis. Seeds of a total of 60 accessions including 15 accessions of each species were taken from Genebank of Plant Genetic Resources Institute, NARC, Islamabad. The germplasm consisted of accessions from different ecological regions of Pakistan.

The accessions were grown in the field of Plant Genetic Resources Institute, National Agricultural Research Center, Islamabad, during Summer 2014, using Augmented Design. Data were recorded according to Bioversity International Descriptor for *Vigna* species (IBPGR, 1985). A total of 8 characters, common for these four *Vigna* species, were recorded in all accessions. These characters included branching pattern, calyx color, corolla color, flowering period, leaf senescence, petiole color, petiole length and twining tendency. Data was subjected to cluster analysis, using Statistica version 7.0 by Ward's method.

Seed Storage Protein analysis of germplasm was conducted, using Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Single seed of each accession was crushed to fine powder by mortar and pestle. 10 mg powder of each sample was mixed with 400 µl protein extraction buffer solution (0.05 M Tris-HCl (pH 6.8), 0.2% SDS, 5 M urea, 10% glycerol, 5% 2-mercaptoethanol and Bromophenol Blue) in 1.5 ml eppendorf tube. Mixture was vortexed and centrifuged at 15000 rpm for 5 minutes. The extracted seed storage proteins were recovered as supernatant. 8% polyacrylamide gel was prepared, using Mini Gel Apparatus AE-6530 of Atto Japan and SDS-PAGE Electrophoresis was conducted as per Laemmli (1970). Data were recorded for the presence and absence of protein bands. Statistica version 7.0 was used for data analysis, using Unweighted pair-group average method.

RESULTS AND DISCUSSION

Cluster analysis of 60 accessions consisting of 15 accessions of *Vigna radiata*, 15 accessions of *Vigna mungo*, 15 accessions of *Vigna aconitifolia* and 15 accessions of *Vigna unguiculata* for qualitative morphological characters resulted into four major clusters using Ward's method (Fig. 1). Cluster I includes accessions of *Vigna aconitifolia*, Cluster II consists of accessions of *Vigna mungo*, Cluster III includes accessions of *Vigna radiata* while all accessions in Cluster IV are of *Vigna unguiculata*.

Zubair *et al*. (2007) reported wide range of variation in Mung bean germplasm on the basis of morphological traits, using cluster analysis. Phylogenetic tree of *Vigna* species studied during the experiment constructed on the basis of morphological characters by cluster analysis is shown in Fig. 2. It indicates a close relationship between *Vigna mungo* and *Vigna radiata*. *Vigna unguiculata* is distantly related to these two species while *Vign aaconitifolia* is different from other three species. Ghafoor *et al*. (2002) described the genetic relationship between *Vigna mungo* and *Vigna radiata* on the basis of morphological traits and SDS-PAGE.

Ghafoor *et al*. (2002) reported that the SDS-PAGE technique can be confidently used to identify inter-specific variation between *Vigna mungo* and *Vigna radiata*. Cluster analysis of germplasm on the basis of Seed Storage Proteins using SDS-PAGE divided the germplasm into 13 major clusters at genetic distance of 1.5 (Fig. 3). Eight clusters (I, V, VI, VIII, IX, X, XI and XIII) had accessions of the same species. Two clusters (II and IV) have accessions of different species but the species are sorted into separate sub-clusters. Cluster III has accessions of *Vigna mungo* and *Vigna unguiculata*, out of which three accessions of *Vigna mungo* and two accessions of *Vigna unguiculata* are in the same sub-cluster. Cluster VII has one accession of *Vigna mungo* and one accession of *Vigna unguiculata* in the same sub-cluster. There are five accessions of *Vigna aconitifolia* and one accession of *Vigna unguiculata* in Cluster XII, out of which one accession of *Vigna aconitifolia* and one accession of *Vigna unguiculata* are in the same sub-cluster.

Simon *et al*. (2007) used DNA amplification fingerprinting (DAF) to estimate the genetic relationships among a representative core collection of cowpea and 16 accessions representing cultivars from 6 *Vigna* species. *Vigna angularis* was found to be the most related, while *V. radiata* the most divergent species relative to *Vigna unguiculata*. Wang *et al*. (2008) used 48 accessions representing twelve *Vigna* species for the study of phylogenetic relationship by molecular markers. The results of cluster analysis showed similar phylogenetic relationships among the *Vigna* species used in the experiment. Cluster analysis divided the accessions into four main clusters and 13 sub clusters. Each sub cluster represented a subgenus or a species.

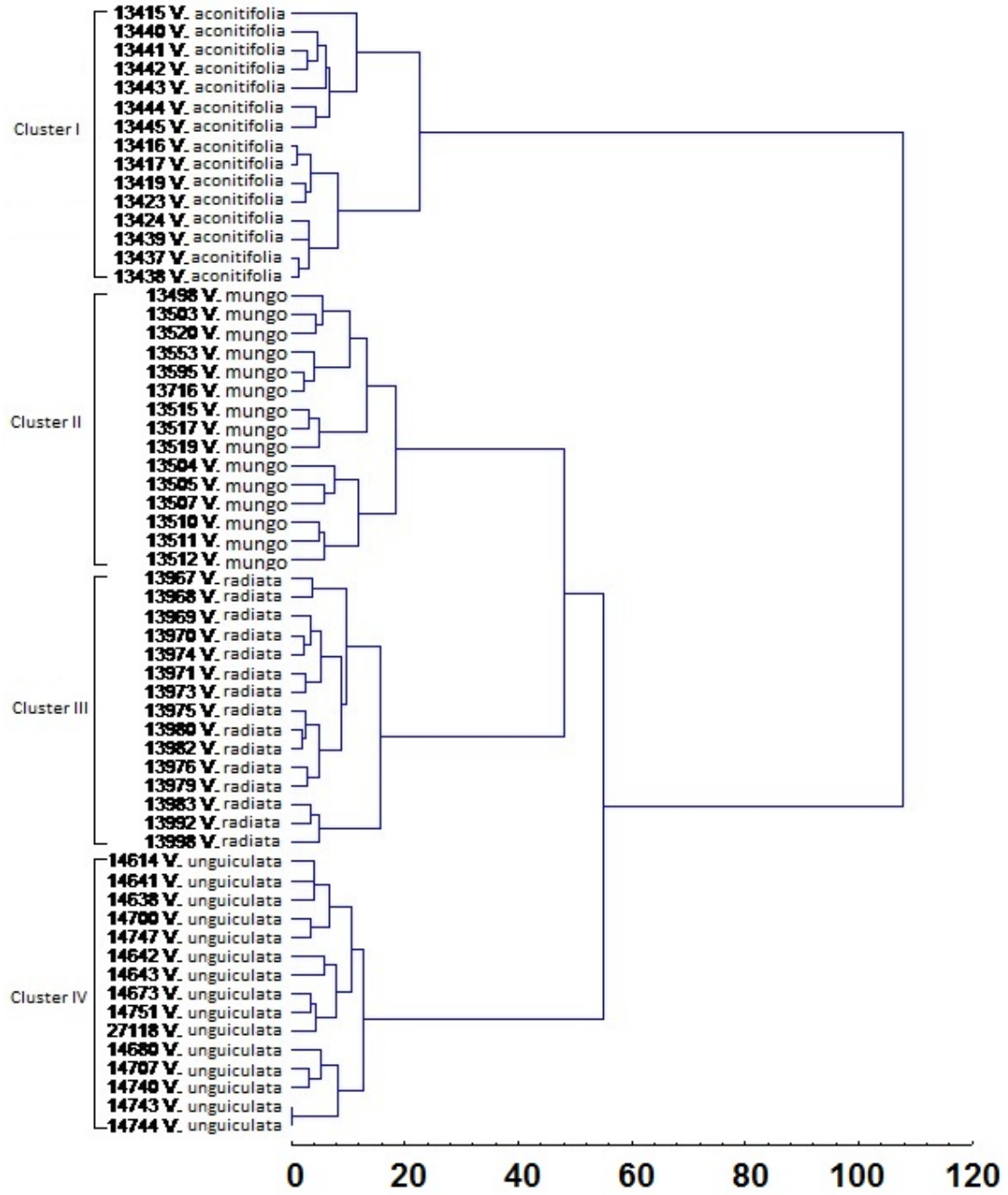

Fig. 1: Cluster analysis of 60 germplasm accessions of *Vigna* species for eight morphological characters, using Ward's method.

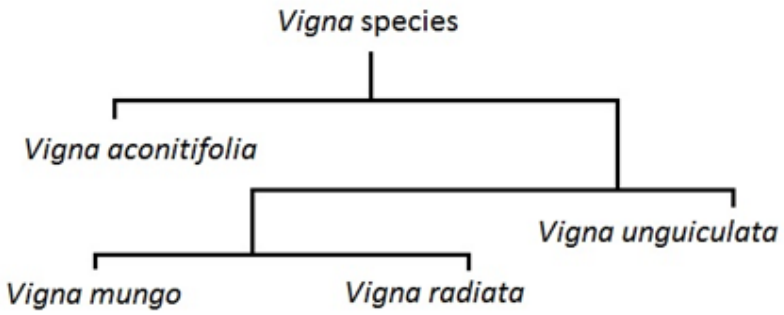

Fig. 2: Phylogenetic tree of *Vigna* species constructed on the basis of cluster analysis using morphological traits studied during the experiment.

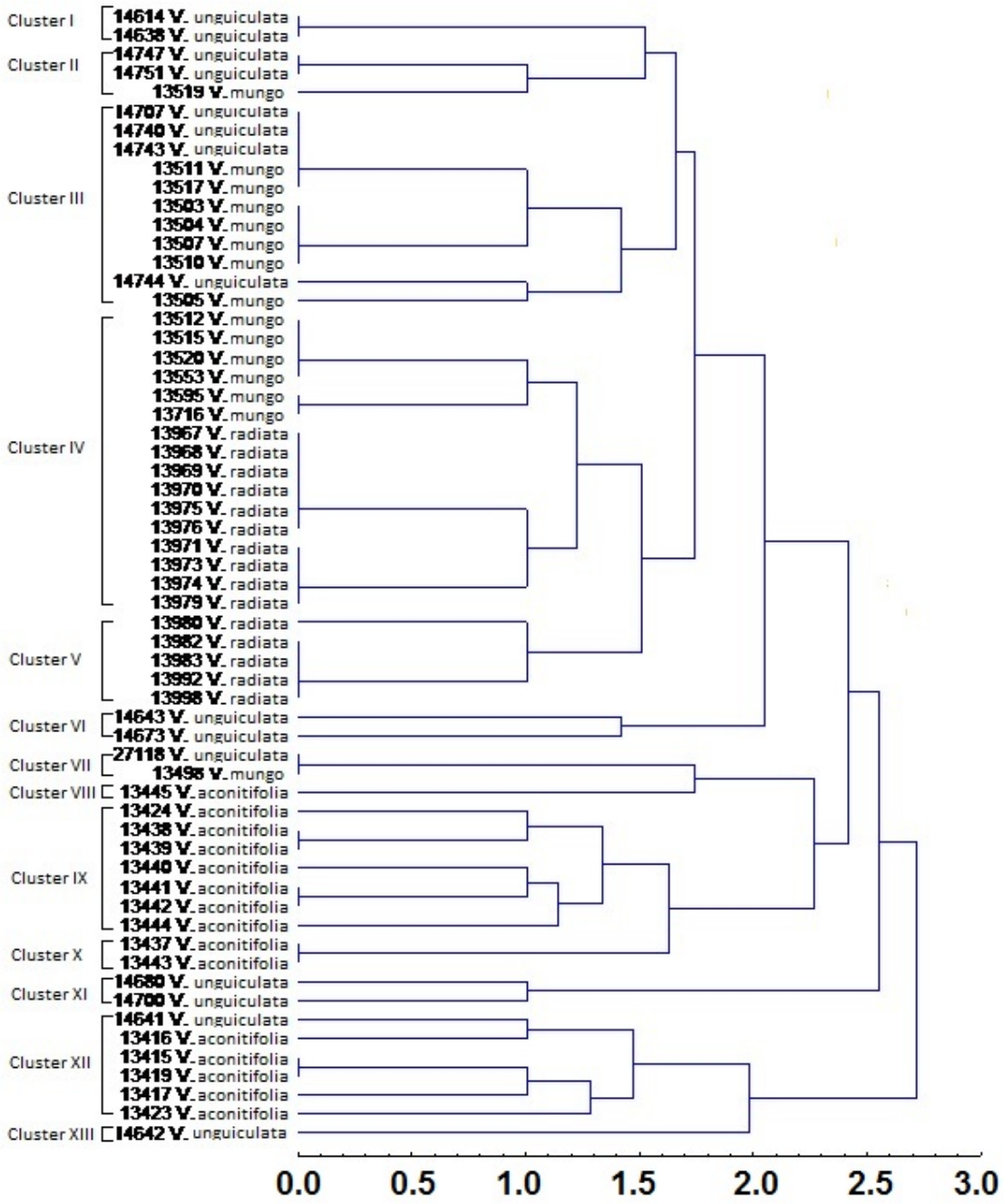

Fig. 3: Cluster analysis of 60 germplasm accessions of *Vigna* species for seed storage proteins, using Unweighted Pair Group Average method.

The results of the experiment show the relationship between four cultivated *Vigna* species. This relationship is helpful for planning the interspecific hybridization for the transfer of desirable traits across the species (Pandiyan *et al*., 2012; Takahashi *et al*., 2016).

The polymorphism, observed for seed storage proteins, shows the genetic diversity within the *Vigna* species. Since, the diversity observed for seed storage proteins categorizes the *Vigna* species in a different way, as compared to the classification on the basis of morphological characters, advanced molecular techniques should be used to review the traditional taxonomic classification (Omonhinmin and Ogunbodede, 2013).

CONCLUSION

The results of experiment show that morphological characters are better than seed storage proteins for taxonomic classification. Cluster analysis for morphological characters indicates that *Vigna radiata* and *Vigna mungo* are in same major cluster at linkage distance of 50 and they are merged with *Vigna* unguiculata at linkage distance of 55. Phylogenetic tree constructed on the basis of morphological characters indicates genetic

relationship among cultivated *Vigna* species that may help to increase the available genepool for crop improvement. Results of seed storage protein analysis give an estimate of protein content diversity and the possible genetic relationship.

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