International Scholars Journals

African Journal of Soil Science ISSN: 2375-088X Vol. 3 (7), pp. 115-122, July, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Diversity of Myanmar cowpea accessions through seed storage polypeptides and its cross compatibility with the subgenus *Ceratotropis*

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Accepted 23 July, 2014

This study was conducted to detect the genetic diversity of sixty eight cowpea (*Vigna unguiculata*) accessions mainly from Myanmar on the basis of protein subunit variations. Fourteen polymorphic bands were recorded out of total 18 resolution bands and the banding pattern revealed four regions. The UPGMA dendrogram showed two distinct clusters. It revealed random grouping of different colored genotypes that indicated no response for discriminating cowpea for different seed-coat color genotypes. Some accessions exhibited a considerable amount of variations although its magnitude was limited through SDS-PAGE 11.25 slab gel. To be broadened, the genetic diversity of cowpea, interspecific hybridization between cowpea and the Asian *Vigna* was conducted. The intermediate band patterns of F1 hybrids were detected from the cross between mungbean and cowpea and thus it confirmed that they were true hybrids by SDS-PAGE analysis and species-specific Simple Sequence Repeat (SSR) primer. It is suggested that SDS-PAGE can also be used as a promising tool to detect genetic relationships of *Vigna* interspecific hybrids because differences were found between known genetic similarities of both parents.

Key words: Cowpea, cross compatibility, genetic relationship, SDS-PAGE, UPGMA dendrogram.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] belonging to the widely cultivated genus *Vigna* is an important crop in many countries of tropical Africa, Asia and South America. It is believed to have originated from Africa (Ng and Monti, 1990), because both wild and cultivated species abound in the region. Cowpea is considered as one of agriculture's oldest legumes used as protein source for people and livestock. Its value lies in its high protein content (23 to 29%), a cheap source of protein for the poor; and its ability to fix atmospheric nitrogen, which allows it to grow on, and improve poor soils (Steele, 1972). The estimated worldwide area under cowpea is about 14 million ha with a production of over 4.5 million tons annually (Singh et al., 2003). Myanmar is regarded as one of the important cowpea growing countries in Asia (Singh and Sharma, 1996) and 7% of the total grain legume is counted as cowpea growing areas.

However, production is constrained by low and variable grain yields, grain quality, susceptibility to diseases and pests and the absence of improved cultivars. Under such

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circumstances, genetic diversity evaluation is of great importance and plays a crucial role in focusing crop improvement. Compared with other leguminous crops, little is known about the population structure of the cowpea and knowledge of patterns of genetic diversity between cultivated and its wild relatives (Vaillancourt, 1993). In order to maintain genetic resources for future sustainable agricultural development, complementary conservation and breeding strategies are necessary (Hawtin et al., 1996). Wide hybridization enables the interspecific gene transfer, which may overcome the narrow genetic variability in the gene pools. Attempts are being made to widen the genetic diversity of cultivated species of Vigna in terms of wide hybridization for the purpose of crop improvement. The introduction of molecular techniques has been made to assess a more accurate evaluation of genetic relationships of cowpea; chloroplast DNA polymorphism (Vaillancourt and Weeden, 1992), restriction fragment length polymorphisms (RFLP) (Fatokun et al., 1993), amplified fragment length polymorphisms (AFLP) (Coulibaly et al., 2002), microsatellite markers (Graham et al., 2001), and random amplified polymorphic DNA (RAPD) (Mignouna et al., 1998; Ghalmi et al., 2010).

However, compared to protein markers, molecular analysis of DNA markers are too expensive. The use of biochemical methods such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique is reliable, inexpensive and without environmental fluctuation. Seed storage protein markers have been successfully used to resolve taxonomic relationships and characterize cultivated varieties in a number of crop plant species; blackgram (Ghafoor and Ahmad, 2005; Ghafoor et al., 2002), *Capsicum annuum* L. (Anu and Peter, 2003), Solanum (Menella et al., 1999), *Vigna* spp (Rao et al., 1992), and wheat (Siddiqui and Naz, 2009), respectively. Proteins are being the end products of gene expression; SDS-PAGE can be employed for various purposes, such as varietal identification, biosystematic analysis, determination of polygenetic relationship between different species and evaluation of passport data (Sammour, 1991).

Furthermore, protein markers have been used as genetic marker to inspect F_1 seed purity of many crops, such as rice (Hong et al., 2006), *Lens* species (Ahmad et al., 1997), and *Brassica* species (Rahman and Hirata, 2004) respectively. There is a limited report of genetic relationship of *V. unguiculata* cultivars based on biochemical analysis in Myanmar. As Myanmar is in the vicinity of the centre of diversity of Vigna, more attention should be paid to the genus Vigna including wild forms and/or landrace varieties for detecting the relationship within and among the specie. In this article, we report on SDS-PAGE analyses of 12 local cultivars, which hold important local adaptation and are of widespread use by farmers throughout the entire country and 56 cultivated accessions provided from seed bank, Myanmar. The aims of the investigation were to examine a) the polymorphism in seed storage proteins within and among *V. unguiculata* cultivar-groups in the collection b) to clarify protein based markers for genotype identification c) to detect cross compatibility of cowpea (*V. unguiculata*) with mungbean cultivar.

MATERIALS AND METHODS

Plant materials

A total of 68 accessions of cowpea (*V. unguiculata*) were evaluated in the present study (Table 1). In addition, one accession of mungbean (*Vigna radiata*) was used for cross compatibility analysis. These accessions were obtained from seed bank, Myanmar.

Genetic relationship estimation on SDS-PAGE analysis

Ten single seeds of each accession were analyzed. For protein extraction, testa-removed seed was ground to a fine powder. Sample buffer (400 µl) was added to 10 mg seed powder as extraction liquid and mixed thoroughly in an Eppendorf tube. The final concentration of the extraction buffer contained 0.5 M Tris-HCl (PH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol blue (BPB) was added to the sample buffer as tracking dye to monitor the movement of protein bands in the gel. Seed protein was analyzed through slab-type SDS-PAGE using 11.25% polyacrylamide gel. Two separate gels were run under similar electrophoretic conditions in order to check the reproducibility of the method. The molecular weights of the dissociated polypeptides were determined using molecular weight protein standards (MW-SDS-70 kit; Sigma, U.S.A.). SDS-PAGE of total protein was carried out in a discontinuous buffer system according to the method of Laemmli (1970). The gels were stained with coomassie brilliant blue (CBB) and then destained until the background was transparent (Masood et al., 2003).

Data analysis

Gels were scored for the presence (1) or absence (0) of every protein band. Only bands showing clear and unambiguous were entered into a data matrix. In order to get clear bands, SDS-PAGE assays were performed more than one time. The unweighted pair group method with arithmetic averages (UPGMA) based on percent disagreement values of the Statistica program were used to construct the matrix and the phylogenetic trees (Statsoft, Ins. Tulsa, USA).

Cross compatibility analysis

The seeds of the genus *Vigna* accessions (cowpea and mungbean) were surface sterilized by dipping in 70% ethanol for 3 min. After washing thoroughly with sterile water, they were grown in pots in a green house at Tokyo University of Agriculture and Technology, Japan. For crossing, selected flowers that are one day prior to opening were emasculated by hands carefully in the early morning. Thereafter, they were pollinated by shaking with the style of male flower accumulating pollen. To prevent evaporation and contamination, a small labeled glycine bags were covered on the candidate flowers. In order to be sure cross combinations and prevent competition among the pods, other flowers and pods on the

Table 1. The name, seed color, seed size, seed luster and source of plant materials.

S. No.	Cultivar name	Seed color	100 Seed weight	Seed luster	Origin
Var.1	Bokate	Grey	26.4	shiny	Seed bank, Myanmar
Var.2	EG-2	Grey	32.35	shiny	Seed bank, Myanmar
Var.3	12 A (Yezin-2)	Reddish brown	26.28	shiny	Seed bank, Myanmar
Var.4	Pe Lun Lonethay	White	16.26	dull	Seed bank, Myanmar
Var.5	Vita-3	Reddish brown	43.99	shiny	Seed bank, Myanmar
Var.6	Unknown	White	10.91	dull	Seed bank, Myanmar
Var.7	TV x 66-2H	Pale brown	25.94	shiny	Seed bank, Myanmar
Var.8	TV x 1193-7D	Pale brown	24.31	shiny	Seed bank, Myanmar
Var.9	TV x 2907-02D	Pale brown	24.94	shiny	Seed bank, Myanmar
Var.10	TV x 1836-0315	Pale brown	36.01	shiny	Seed bank, Myanmar
Var.11	IT-82E-32	Pale brown	31.61	shiny	Seed bank, Myanmar
Var.12	Cowpea174	Pale purple	26.79	shiny	Seed bank, Myanmar
Var.13	TV x 3871-02F	Pale brown	39.96	shiny	Seed bank, Myanmar
Var.14	TV x 4654-44E	Pale brown	33.16	shiny	Seed bank, Myanmar
Var.15	TV x 4677-010E	White	49.37	dull	Seed bank, Myanmar
Var.16	IT-81D-1064	Reddish brown	44.54	shiny	Seed bank, Myanmar
Var.17	Black Eye Bean	White	40.55	dull	Seed bank, Myanmar
Var.18	Hulli Cowpea	Pale brown	27.91	shiny	Seed bank, Myanmar
Var.19	IT-82E-18	Pale brown	32.31	shiny	Seed bank, Myanmar
Var.20	IT-82D-885	Pale purple	41.18	shiny	Seed bank, Myanmar
Var.21	IT-82D-716	Spotted	13.746	shiny	Seed bank, Myanmar
Var.22	IT-82D-752	Pale brown	30.18	shiny	Seed bank, Myanmar
Var.23	IT-82D-786	Ochre	29.7	shiny	Seed bank, Myanmar
Var.24	Cowpea (Red)	Red	29.64	shiny	Seed bank, Myanmar
Var.25	Ta La Pe	stripe	20.98	shiny	Seed bank, Myanmar
Var.26	IT-82D-699	Brown	26.34	shiny	Seed bank, Myanmar
Var.27	IT-83D-328-5	Pale brown	34.35	shiny	Seed bank, Myanmar
Var.28	IT-84D-460	Ochre	34.96	shiny	Seed bank, Myanmar
Var.29	IT-85F-2020	Ochre	40.74	shiny	Seed bank, Myanmar
Var.30	IT-85F-2076	Spotted	40.34	shiny	Seed bank, Myanmar
Var.31	Vu-87-28	Pale purple	14.92	shiny	Seed bank, Myanmar
Var.32	Vu-87-36	Deep purple	13.89	dull	Seed bank, Myanmar
Var.33	Vu-89-7	Creamy	15.66	shiny	Seed bank, Myanmar
Var.34	Vu-89-9	Creamy brown	9.136	dull	Seed bank, Myanmar
Var.35	Vu-90-4	Deep grey	10.56	shiny	Seed bank, Myanmar
Var.36	Boma	White	42.81	dull	Seed bank, Myanmar
Var.37	Hleku Bokate	Brownish grey	21.71	shiny	Seed bank, Myanmar
Var.38	IT-82E-16	Brown	15.26	shiny	Seed bank, Myanmar
Var.39	IT-82E-9	Black	36.72	shiny	Seed bank, Myanmar
Var.40	IT-85F-867	Brown	28.84	shiny	Seed bank, Myanmar
Var.41	Vu53	Grey	10.3	shiny	Myanmar, collection
Var.42	IT-82D-744	Pale brown	28.96	shiny	Seed bank, Myanmar
Var.43	vu102002-04	Stripe	17.3	shiny	Myanmar, from farmer
Var.44	vu21	Brown	13.27	shiny	Myanmar, from farmer
Var.45	Vu6-2003-04	Stripe	20.66	shiny	Myanmar, from farmer
Var.46	Vu14-2003-04	Stripe	16.64	shiny	Myanmar, from farmer
Var.47	Pe Lun Phyu	White	20.26	dull	Myanmar, from farmer
Var.48	Vu13FARV	Ochre	30.82	shiny	Seed bank, Myanmar
Var.49	IT84E124	Pale brown	33.34	dull	Seed bank, Myanmar
Var.50	Vu15Vu87-30	Pale brown	12.44	shiny	Myanmar, from farmer
Var.51	Redcowpea6	Deep brown	11.51	shiny	Myanmar, from farmer

Table 1. Contd.

Var.52	Vu12S2	Stripe	15.66	shiny	Myanmar, from farmer
Var.53	Vu92003-04	Reddish grey	11.31	shiny	Myanmar, from farmer
Var.54	Vu8Vu89-22	Brown	16.55	shiny	Seed bank, Myanmar
Var.55	Vu7Vu89-T	Creamy	14.72	shiny	Myanmar, from farmer
Var.56	Vu4IT82D789	Pale brown	28.45	shiny	Seed bank, Myanmar
Var.57	Vu3IT81D1020	Deep brown	12.42	shiny	Seed bank, Myanmar
Var.58	Vu3S1	White	23.7	dull	Myanmar, from farmer
Var.59	IT85F-1380	Ochre	27.74	shiny	Seed bank, Myanmar
Var.60	IT82D-812	Pale brown	30.81	shiny	Seed bank, Myanmar
Var.61	Vu152003-04	Stripe	15.1	shiny	Myanmar, from farmer
Var.62	Vu16C1	White	22.9	dull	Seed bank, Myanmar
Var.63	VuC2BEP	White	14.02	dull	Myanmar, from farmer
Var.64	Vu29-2003-04	White	12.964	dull	Myanmar, from farmer
Var.65	89IT82D-889	Deep brown	29.27	shiny	Seed bank, Myanmar
Var.66	TV x 289-4G	Pale brown	28.3	shiny	Seed bank, Myanmar
Var.67	IT85F-1517	Creamy	26.56	dull	Seed bank, Myanmar
Var.68	Vu89-3	Brown	16.17	shiny	Myanmar, from farmer

Seed bank, Myanmar – the accessions provided by Seed bank, Myanmar. Myanmar, from farmer – the accessions collected from farmers' fields throughout the country.

inflorescence were removed. Pod setting was observed 4 to 5 days after pollination. Pods were harvested at maturity.

Analysis of DNA using microsatellite markers

Total DNA from green leaves (bulk sample) was extracted by the rapid DNA extraction method using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). PCR (ASTEC, Gene Amp PC system 320, Japan) was performed with 25 µl of reaction components including 15.4 µl of double distilled water (DDW), 1 unit of Taq DNA Polymerase (Takara, Japan), 20 ng of genomic DNA, 2.5 µl of 10X buffer (Fermentas Inc®), 2 µl of 50 mM $MgCl_2$, 2 µl of DNTPs [5 mM], 10 pM VM37 primer, (forward) 5`TGT CCG CGT TCT ATA AAT CAG C and Reverse: 5`CGA GGA TGA AGT AAC AGA TGA TC to distinguish between parents and their hybrid (Figure 1). The PCR conditions were as follows: denaturation at 94°C for 1 min followed by 30 cycles at 94°C for 30 s, at 58°C for 30 s, at 72°C for 30 s, and final elongation step at 72°C for 10 min. PCR products were separated by electrophoresis on a 1% agarose gel.

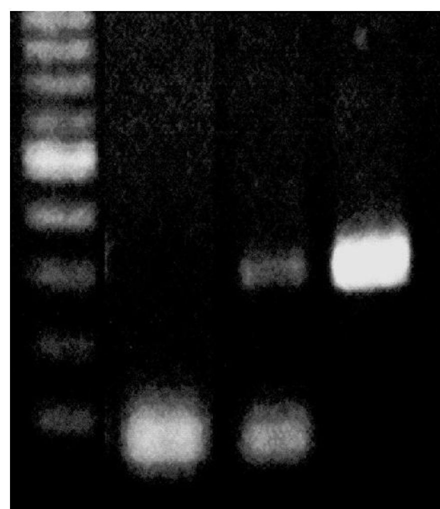

Figure 1. Amplification profile of Vigna parents and their F1 with polymorphic SSR primer (VM 37), M: Marker (DNA ladder), P1: mungbean, P2: cowpea, F1: First generation hybrid.

RESULTS

Diversity of electrophoric profile of seed storage protein of cowpea accessions

The band patterns of cowpea accessions in this study are shown in Figure 2. Seed storage proteins of 68 accessions of cowpea examined by SDS-PAGE in the present study produced 18 resolution bands with apparent molecular weight ranging from 97 to 15 kDa and they were used to classify cowpea germplasm. Out of 18 protein bands, of which 14 were polymorphic and 4 were monomorphic. The polypeptide bands vary considerably with respect to their staining intensities in all the varieties. In this study, variations in the polypeptide bands among cowpea varieties were observed to be more of staining intensity than of presence or absence. For example, the varieties of Vu-87-28, IT-82D-744 and Vita 3 (lanes 4, 7, and 9) can be distinguished by differences in the staining intensities of band of apparently molecular weight

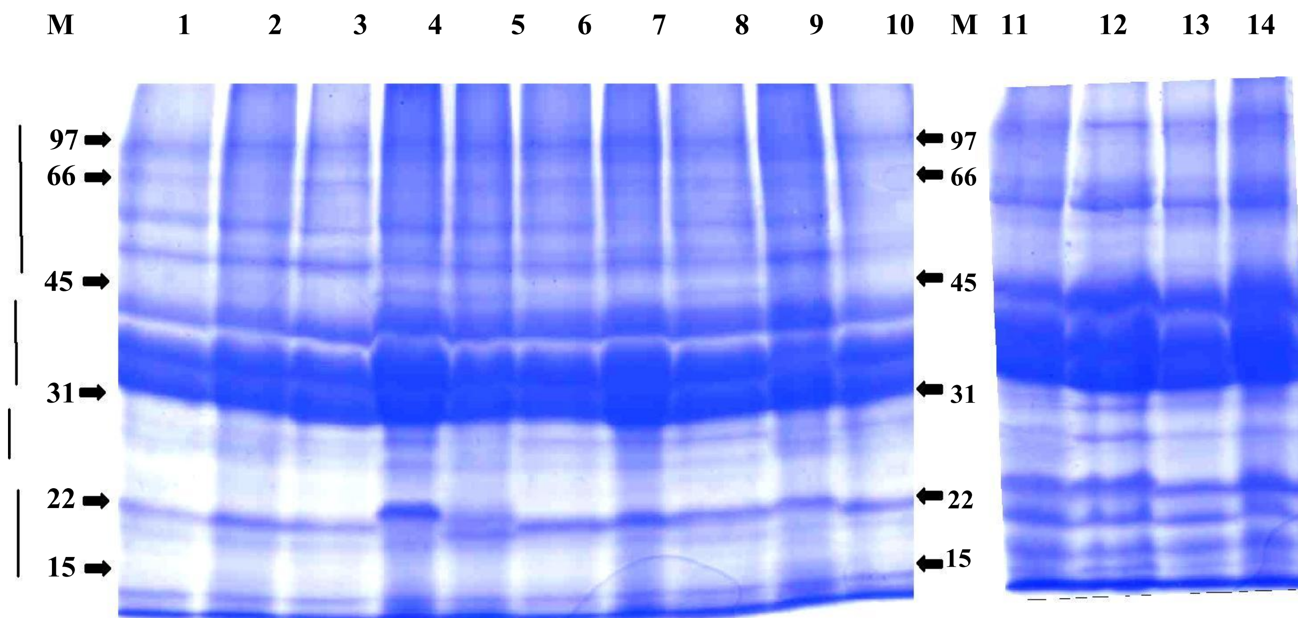

Figure 2. Electrophoregram of bulk representative samples of studied cowpea accessions. Lanes 1 to 14 refer to the cultivars Vu21, IT-82E-9, TV x 1193-7D, Vu-87-28, IT-85F-2076, Vu53, IT-82D-744, Vu10-2002-04, Vita-3, IT-82E-16, Pe Lun Phyu, Boma, Vu87-30, and FARV, respectively. M -marker. Total seed proteins extracted from equal amounts of seed powder from each genotype were separated by SDS-PAGE using a 11.25% acrylamide gel and stained with Coomassie Blue.

between 45 and 31 kDa, respectively (Figure 2). The presence or absence of some bands and differences in staining intensities of bands are useful to separate the varieties into distinguishable groups (Odeigah et al., 1999).

The banding patterns within an accession were repeatable with different seed lots and results showing some intra-variety differences were observed. On the basic of banding patterns, the stained gel is divided into 4 regions, A, B, C, and D (Figure 2). The region A had the bands between 97 to 45 kDa MW of which 4 out of 6 were polymorphic. The high degree of variations was observed in this region. The region B ranged from 45 to 31 kDa having 3 bands of which 2 were monomorphic. The high intensity bands were detected in this region and majority of the genotype showed similar banding patterns. Molecular weight between 31 and 20 kDa were recorded as region C having 2 specific bands. Seven protein peptides ranging from 22 to 15 kDa were included in the region D. Most of the genotypes showed a considerable amount of variations in this region.

Cluster analysis

A dendrogram of the 68 cowpea accessions was constructed by the unweighted paired group method using arithmetic averages on the basis of percent disagreement values (Figure 3). Protein subunit variations using UPGMA showed that the accessions were clustered as two distinct groups, A and B. Percent disagreement values based on protein banding patterns of 68 accessions ranged from 0 to 0.45. Cluster A composed of a maximum of 45 accessions showing the highest number of repeated genotypes (genetic similarity to each other).

Cluster B comprised of 23 genotypes of which most of them are gene bank accessions. The various seed color genotypes are scattered into both clusters with regardless of growing regions. The relatively high genetic dissimilarity was found in the genotype with black colored seed (Var.39) at 0.23 PDV with other genotypes in cluster A. The results of principle component analysis and cluster analysis were in agreement for the material used in this study (Figure 4). Up to 73% of the total variations was explained by the first three axes, which accounted respectively for 44.51, 21.25 and 7.92% of the observed variations. Accession (Var. 39) showed more difference from other genotypes in group A.

Cross compatibility between cowpea and mungbean

Cross combination ability between the subgenus *Ceratotropis* (mungbean) and *Vigna* (cowpea) was observed in terms of interspecific hybridization.

Cross combination between mungbean and cowpea

This cross was only successful when mungbean (*V.*

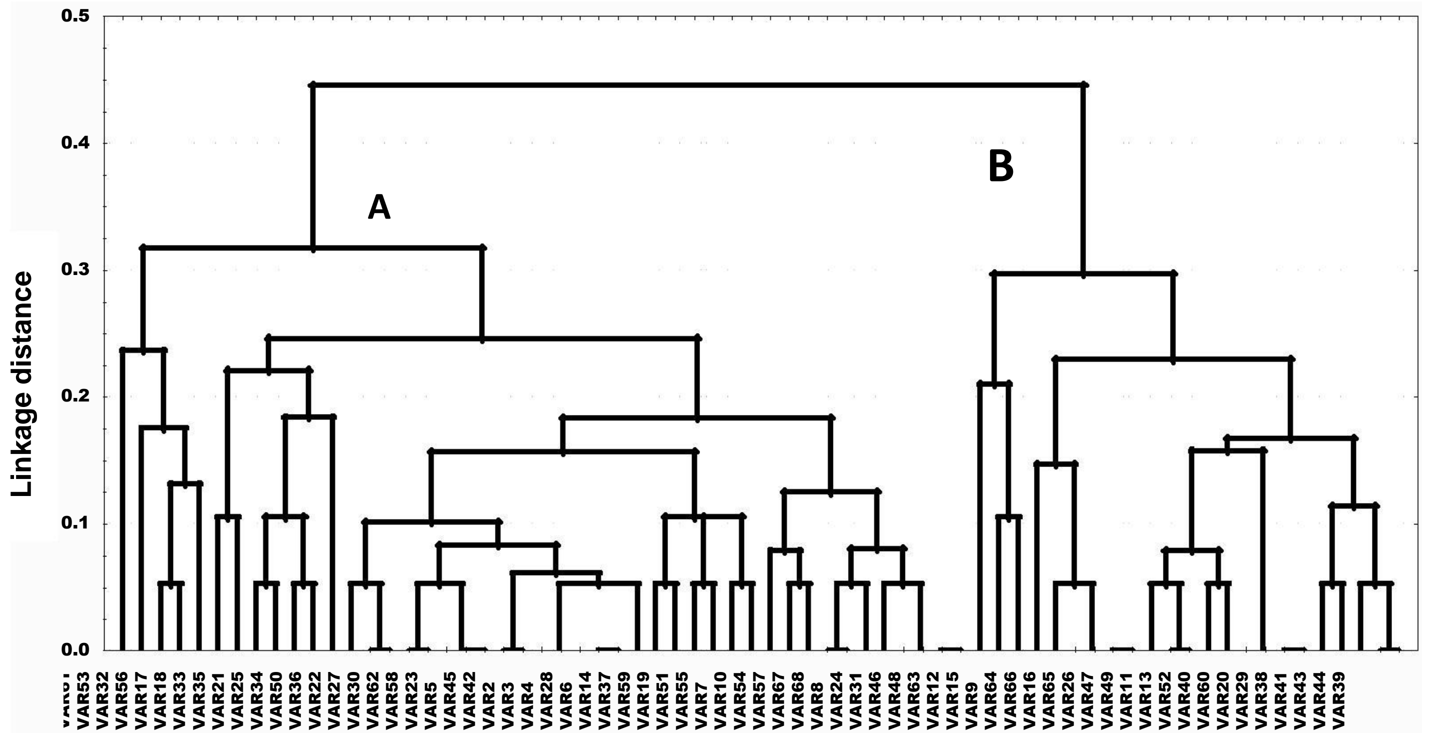

Figure 3. The dendrogram based on seed protein banding patterns in bulked seed samples representing the genetic relationships among the 68 cowpea accessions. (Code numbers of the accessions were listed in Table 1).

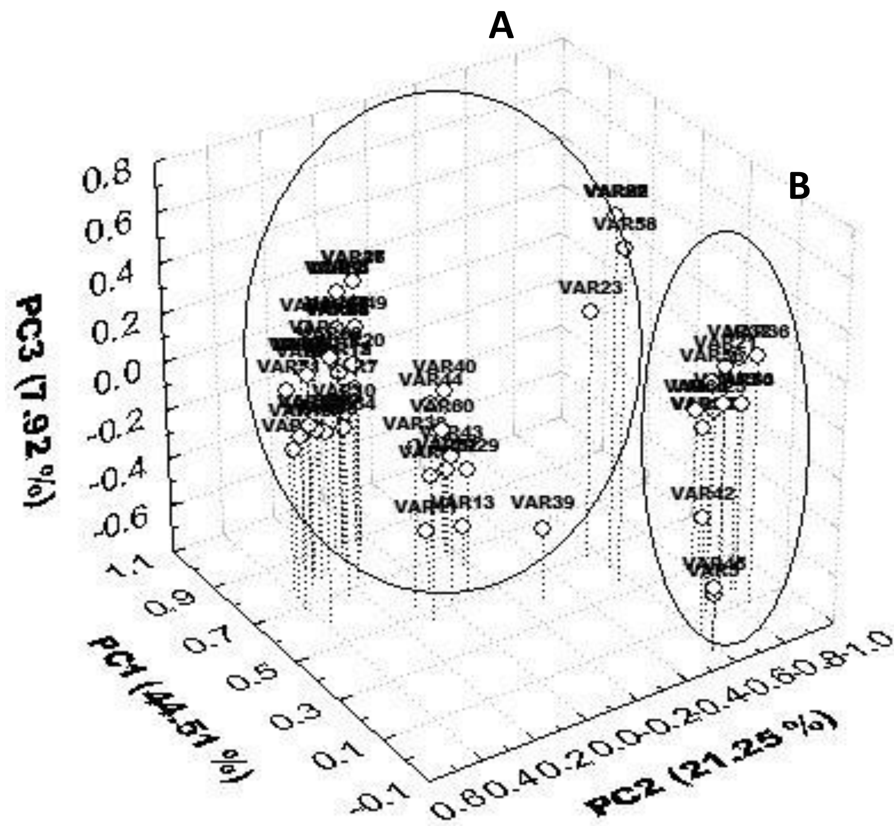

Figure 4. Three-dimension principle coordinate analysis of the 68 cowpea accessions (Code numbers of the accessions were listed in Table 1).

radiata) was used as seed parents. However, pod setting showed the lowest and the highest rate of young pod abortion between 3 to 10 days after pollination. Only (1.02%) of pod setting was observed and finally, we obtained the viable seeds from the cross (mungbean x cowpea). The obtained F_1 seeds were subjected to banding patterns by SDS-PAGE analysis and analysis of DNA using microsatellite markers. The reciprocal crosses have never succeeded.

Banding nature of F_1 of mungbean and cowpea cross

Similar and different banding patterns between F_1 and its parents (mungbean x cowpea) were detected by SDS-PAGE analysis (Figure 5). Electrophoretic profile of F_1 shared the banding patterns with its parents. The bands on position (a) were observed as similar patterns with their pollen parent (cowpea). More staining band profiles were observed in position (b). The position (c and d) bands were very close to by pollen parents. Exception of more band intensity on F_1, banding patterns of (e and f) positions were very similar with its seed parent (mungbean). The (g) position band showed intermediate pattern between both parents. New banding patterns were detected in (h and i) positions of F_1 seeds. So, it was observed that Hybrid individual had extra bands addition to both parental bands. Moreover, compared to both parents, high staining intensity bands were observed in F_1 hybrid.

To prove the true hybrid between mungbean and cowpea by SDS-PAGE, it was also chased by molecular level confirming that it was true hybrid. The seeds of both parents and F1 were germinated under glasshouse conditions. These seedlings (5 sample leaves from each in bulk population) examined by the species- specific SSR marker, VM37, seedlings were found to be hybrids by sharing the bands of both parents (Figure 1). VM37 microsatellite primer sets designed from the sequences of cowpea was able to amplify DNA of mungbean and F_1 hybrid of mungbean and cowpea. Therefore, microsatellite markers of cowpea could be used to detect F_1 hybrid purity and comparative genome analysis between the different *Vigna* species.

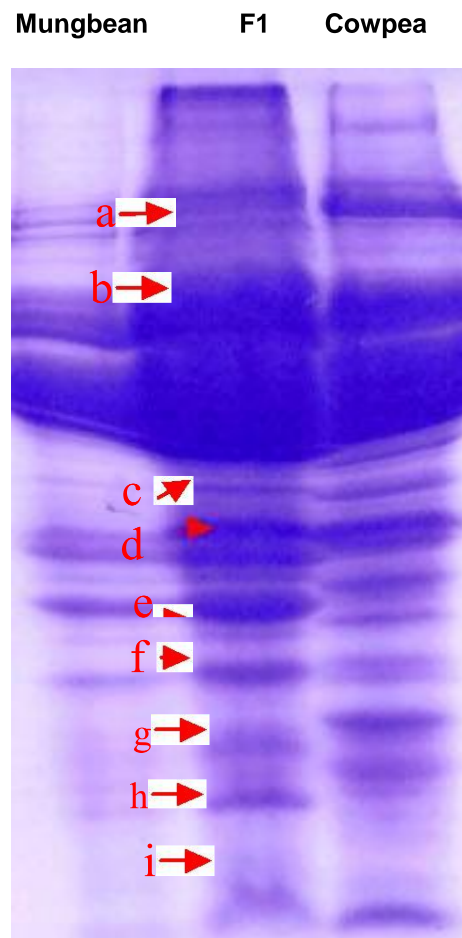

Figure 5. SDS-PAGE analysis of total seed proteins of parental lines and their hybrid; Total seed proteins extracted from equal amounts of seed powder from each genotype were separated by SDS-PAGE using a 11.25% acrylamide gel and stained with Coomassie Blue.

DISCUSSION

Characterization of diversity among genotypes is of paramount importance to crop improvement programs. In this investigation, 68 accessions of cowpea revealed a considerable level of variations among the accessions through biochemical markers. The repeated genotypes in the cluster analysis mean these genotypes have similar and identical electropharegrams and showing no genetic variation in protein subunits. Similar and different patterns of protein electrophoregram could be used as passport data in Myanmar seed bank for the future breeding improvement. In addition for better management of the gene bank, precise and comprehensive knowledge of agricultural and biochemical data (protein and DNA) is essential so that duplicates can be eliminated; this will help in compiling a core collection of cowpea germplasm. Variations in SDS-PAGE can be exploited to understand the extent of genetic diversity and the relationship among Myanmar cowpea accessions. Genotypes with similar banding patterns have been suggested to be further studied for detailed agronomic and biochemical analyses, including 2-D electrophoresis and DNA markers, for better management of the gene bank (Celis and Bravo, 1984; Beckstrom-Sternberg, 1989).

Interspecific hybridization is one of the useful ways for

transferring the desirable traits from one species to another and promoting the increase of genetic variability. Cross compatibility analysis is a prerequisite tool for interspecific hybridization because the species which are closely related have high possibility of pod setting and ability of gene transfer. This study reveals that the cross between mungbean and cowpea was successful although pod setting percentage was low and minimum. This interspecific cross is of great potential for use in genus Vigna breeding improvement for increase genetic variability of the populations of cultivated species. The failure of interspecific hybridization (reciprocal crosses) of cowpea and mungbean might be involved the pollen tubes are unable to penetrate the stigma and style (Chowdhury and Chowdhury, 1977), due to young embryo degradation (Ahn and Hartmann, 1978; Fatokun, 1991) and F_1 interspecific hybrids may be completely sterile (Chen et al., 1983).

After pre-fertilization, post-fertilization barriers might hinder the development of embryo and formation of endosperm (Gopinathan et al., 1986). Post-fertilization barriers can be overcome under certain circumstance, such as embryo rescues (Agnihotri, 1993), or application of plant growth regulators (Mallikarjuna, 1999), however, the responses of these attempts varied in different crosses. SDS-PAGE analysis was used to detect different and similar protein banding patterns between F_1 (mungbean x cowpea) and their parents for analyzing whether true F_1 or self pollinated plants. Banding patterns of mungbean x cowpea hybrid showed combined patterns of their maternal and paternal parents and confirming that it is true hybrid. To our knowledge, except for a few reports of successful crosses between mungbean and cowpea (Stanton, 1964; Tyagi and Chawla, 1999), the information on successful interspecific hybrids between them is still limited. The study on genome analysis of mungbean and cowpea using RFLP mapping data indicated that a high degree of similarity in the nucleotide sequences among these species (Young et al., 1993). In conclusion, this investigation showed that:

1) A considerable level of variations were detected among the cultivated cowpea from different regions of Myanmar by SDS-PAGE analysis and this information would help us to gain an insight into cowpea divergence which can be exploited for hybridization;

2) Interspecific hybridization between the subgenus *Ceratotropis* (mungbean) and *Vigna* (cowpea) is possible because it is proved that F_1 hybrid was observed between the cross of mungbean and cowpea; it has been possible, therefore, to transfer genes from cowpea to mungbean; and

3) This research confirmed that protein banding patterns of both parents compared with their respective hybrid clearly recognized true hybrids, and SDS-PAGE could be regarded as a promising tool to detect interspecific hybrid in the genus *Vigna*.

ACKNOWLEDGEMENTS

The research reported in this work was funded by the Japanese Government (Monbukagakusho:Mext) Scholarship Association. The authors express their genuine thanks to the Myanmar Seed bank for furnishing much of the material used in this study.

REFERENCES

Agnihotri A (1993). Hybrid embryo rescue. In: Lindsey, K., (eds.) Plant Tissue Culture Manual: Fundamentals and Applications, Kluwer Academic Press, Dordrecht, Boston, London, pp. 1-8.

Ahmad M, Fautrier AG, Burritt DJ, Mcneil DL (1997). Genetic diversity and relationships in *Lens* species and their F1 interspecific hybrids as determined by SDS-PAGE. New Zeal. J. Crop Hort., 25: 99-108.

Ahn CS, Hartmann RW (1978). Interspecific hybridization between mung bean (*Vigna radiata* [L.] Wilczek) and adzuki bean (*V. angularis* [Wild.] Ohwi and Ohashi). J. Am. Soc. Hortic. Sci., 103: 3–6.

Anu A, Peter KV (2003). Analysis of seed protein of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis. Genet. Resour. Crop Evol., 50: 239–243.

Beckstrom-Sternberg SM (1989). Two-dimensional gel electrophoresis as a taxonomic tool: evidence from the *Centrospermae*. Biochem. Syst. Ecol., 17: 573–582.

Celis JE, Bravo R (1984). Two dimensional gel electrophoresis of proteins; methods and applications. Academic Press, New York.

Chen NC, Baker LR, Honma S (1983). Interspecific crossability among four species of *Vigna* food legumes. Euphytica, 32: 925–937.

Chowdhury RK, Chowdhury JB (1977). Intergeneric hybridization between *Vigna mungo* L. Hepper and *Phaseolus calcaratus* RoxB. Indian J. Agric. Sci., 47: 117–121.

Coulibaly S, Pasquet RS, Papa R, Gepts P (2002). AFLP analysis of the phonetic organization and genetic diversity of *Vigna unguiculata* L. Walp. reveals extensive gene flow between wild and domesticated types. Theor. Appl. Genet., 104: 358–366.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bull., 19: 11-15.

Fatokun C, Danesh D, Young ND (1993). Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. Theor. Appl. Genet., 86: 97–104.

Fatokun CA (1991). Wide hybridization between *Vigna pubescens* and *V. unguiculata* through embryo rescues. Plant Cell Tissue Organ. Cult., 9:229–233.

Ghafoor A, Ahmad Z, Qureshi AS, Bashir M (2002). Genetic relationship of *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilezek based on morphological trats and SDS-PAGE. Euphytica, 123: 367-378.

Ghafoor A, Ahmad Z (2005). Diversity of agronomic traits and total seed protein in blackgram *Vigna mungo* (L.) Hepper. Acta Biologia Cracoviensia Series Botanica, 47(2): 69–75.

Ghalmi N, Malice M, Jacquemin J-M, Ounane S-M, Mekliche L, Baudoin J-P (2010). Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata* (L.) Walp.) landraces. Genet. Resour. Crop Evol., 56: 299-310.

Gopinathan MC, Babu CR, Shivanna KR (1986). Interspecific hybridization between rice bean (*Vigna umbellata*) and its wild relative (*V. minima*): Fertility-sterility relationships. Euphytica, 35: 1017–1022.

Graham JS, Cheng DL, Fatokun CA, Benjamin U, Singh BB (2001). Determining Genetic Similarities and Relationships among Cowpea Breeding Lines and Cultivars by Microsatellite Markers. Crop Sci., 41: 189–197.

Hawtin G, Iwanaga M, Hodgkin T (1996). Genetic resources in breeding for adaptation. Euphytica, 92: 255–266.

Hong JW, Na L, Hong DL (2006). Genetic diversity of seed storage proteins in different ecotype varieties of japonica rice and its application. Rice Sci., 13: 85–92.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680–685.

Mallikarjuna N (1999). Ovule and embryo culture to obtain hybrids from interspecific incompatible pollination in chickpea. Euphytica, 110: 1–6.

Masood S, Ghafoor A, Javaid A (2003). Laboratory manual characterization and evaluation of plant genetic resources. Plant Genetic Resources Institute, NARC, Islamabad, Pakistan.

Menella G, Sanaja VO, Tonini A, Magnifico V (1999). Seed storage protein characterization of *Solanum* sps. and of cultivars and androgenetic lines of *S. melogena* L. by SDS-PAGE and AE-HPLC. Seed Sci. Technol., 27: 23-35.

Mignouna HD, Ng NQ, Ikca J, Thottapilly G (1998). Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. J. Genet. Breed., 52: 151–159.

Ng NQ, Monti LM (1990). Cowpea Genetic Resources. International Institute of Tropical Agriculture, Ibadan, Nigeria, p. 200.

Odeigah PGC, Ohoh B, Aghalokpe IO (1999). The characterization of Nigerian varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* by SDS-polyacrylamide gel electrophoresis of seed proteins. Genet. Resour. Crop Evol., 46: 127–131.

Rahman MM, Hirata Y (2004) Genetic diversity in *Brassica* species using SDS-PAGE analysis. J. Biol. Sci., 4: 234–238.

Rao R, Vagliol MD, D'Urzo MP, Month L (1992). Identification of *Vigna* spp. Through specific seed storage polypeptides. Euphytica., 62: 39-43.

Sammour RH (1991). Using electrophoretic techniques in varietal identification, biosystematic analysis, phylogentic relations and genetic resources management. J. Islamic Acad. Sci., 4: 221–26.

Siddiqui MF, Naz N (2009). Protein landmarks for diversity assessment in wheat genotypes. Afr. J. Biotechnol., 8(9): 1855-1859.

Singh BB, Ajeigbe HA, Tarawali SA, Fernandez-Rivera S, Abubakar M (2003). Improving the production and utilization of cowpea as food and fodder. Field Crops Res., 84: 169–177.

Singh BB, Sharma B (1996). Restructuring cowpea for higher yield. Indian J. Genet., 56: 389–405.

Stanton WR (1964). Draft report on the work of grain legume section for 1961. Rep. Res. Stn. Min. Agric. Sawan, Nigeria.

STATSOFT INC (2001). STATISTICA data analysis software system, version 6. Tulsa, Oklahoma.

Steele WM (1972). Cowpea in Africa. Doctoral thesis. University of Reading, United Kingdom.

Tyagi D, Chawla HS (1999). Effects of seasons and hormones on crossability barriers and *in vitro* hybrid development between *Vigna radiata* and *Vigna unguiculata*. Acta Agronimica Hungarica, 47: 147–154.

Vaillancourt RE (1993). Isozyme diversity in the cowpea species complex. Crop Sci., 33: 606-613.

Vaillancourt RE, Weeden NF (1992). Chloroplast DNA polymorphism suggests a Nigerian centre of domestication for the cowpea *Vigna unguiculata* (Leguminosae). Am. J. Bot., 79: 1194–1199.

Young ND, Menancio-Hautea D, Fatokun CA, Kumar L, Danesh D (1993). Comparative genome analysis of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*V. unguiculata* L. Walpers) using RFLP mapping data. Theor. Appl. Genet., 86:797-810.