DOES BIOMASS HAVE IMPACT ON SELECTING *VIGNA MUNGO* FOR ORGANIC FARMING UNDER RAIN-FED CONDITIONS?

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Abstract

Under emerging challenges of climate change and enhanced demands for organic vegetable protein, black gram is a potential commodity that can successfully be grown with zero inputs due to favorable genes eco-friendly agricultural system. Seventy seven genotypes evaluated for three consecutive years indicated variability attributed genetic differences. The clustering patterns were affected by environmental effects that were further determined by principal components. Although performance for the germplasm was affected by the year effects, especially for maturity duration, however on the basis of average performance, 11 genotypes were identified and among these 45304, 45920 and 45921) were earlier than the check and produced considerably high grain yield. Selected genotypes are suggested for future testing under wide range of agro-ecological regions. It was obvious that the selected genotypes exhibited higher biomass that was partitioned to the economic yield, it should the selection criterion for suitable cultivars under rain-fed condition. Breeding program can utilize selected genotypes to improve black gram for organic farming.

Key words: *Vigna mungo,* Biomass, Organic farming, Rain-fed.

Introduction

Vigna mungo (L.)Hepper commonly known as mash, urd or black gramis a summer food legume that is being cultivated widely in the tropics as well as sub-tropics including Asia, Africa and South America (Kumar *et al.,*2012). It has been identified as a potential source for dietary protein, minerals and vitamins for poor masses of the poor part of the world (Singhal *et al.,* 2014). Better agronomic performance, especially for seed yield attributes, successive selection and the probable linearity between biomass and economic yield is the most important selection criteria for future elite cultivars of crop plants (Ḋhoop *et al.,* 2014; Gupta *et al.,* 2016). Black gram germplasm has been exclusively investigated for genetic diversity and its utilization in genetic improvement (Ghafoor *et al.,* 2001; Ghafoor & Arshad, 2011). Recent initiatives for improvement of crop germplasm for eco-friendly agricultural production have also been taken by different breeding programs (Ilyas *et al.,* 2014). Organic agriculture was the most primitive farming that man started during early era of human civilization, but later polluted by intervention of inorganic chemicals to have maximum production for food production, but at health risk (Singhal *et al.,* 2014). Billions of USDs were earned through incorporation of inorganic fertilizers and contract farming. Recent concept of health food has made shift over to green food that has altered crop breeding strategies to produce chemical free food, especially in the developed world where public is more health sentient (Patil *et al.,* 2016). Due to high atavistic potential of legumes, *Vigna mungo* is expected to exhibit desirable diversity for selecting cultivars suitable for organic farming under rain-fed conditions (Tripathy *et al.,* 2016).

Magnitude of genetic diversity particularly having linearity with additive gene effects is expected to be exploited through simple selection under the environments where the experiments are conducted (Bonneau *et al.,* 2013). In particular, the anticipated climate change and the emerging demand for organic food has ridiculously diverted the breeding approaches to develop suitable cultivars for the coming decades to ensure healthy food for all to save the globe, particularly when modern cultivars do not possess favorable alleles for challenges of future agricultural productivity. Keeping in view the emerging challenges of climate change and the need for organic farming, the blackgram germplasm was evaluated under rain-fed conditions under zero inputs for inorganic chemical to estimate genetic potential coupled with diversity for selecting suitable cultivars that will enable us to adopt suitable breeding programs to cope the demands of food security in the coming decades. As *Vigna mungo* has been originated and diversified in the Indian subcontinent (Sehrawat & Yadav, 2014), and huge variation has been reported in the indigenous genetic resources, hence scope for selecting desirable genotypes suitable for eco-agricultural system is expected at high probability levels.

Materials and Methods

Indigenous blackgram genetic resources (collected from Pakistan) comprising of 77 genotypes including Mash 1 as check variety were evaluated for days to maturity, biomass plant[-1], grain yield plant[-1] and harvest index (%) during three consecutive years, *i.e.,* 2011, 2012 & 2013. Augmented design was applied and the experiment was planted at National Agriculture Research Center (NARC), Islamabad, Pakistan. Similar specifications were kept all the three years for experimentation, except yearly change in trial location to avoid mixing from left over seed of previous year, but the broadly the field where experiments were conducted was the same. Four rows of 4 m length were planted with inter-row and intra-row spacing of 30 and 10 cm, respectively and the check variety was planted after every seven entries (repeated 10 times) during all the three years. The experiments were

kept chemical free and no irrigation was applied but only under rain-fed conditions. The area under experiments remained free from inorganic applications; therefore evaluation under specific microclimatic conditions will enable us to identify the actual potential under rain-fed organic farming. Twenty competitive plants were sampled within each genotype at maturity for data recording on biomass and grain yield, however days to maturity were taken on line basis that was presented by single value and harvest index was expressed as economic yield over total biomass production in percentage.

Analysis of variance for estimation of differences among genotypes as well as years was performed along with basic statistics with the help of computer software "MS-Excel. The genetic parameters (braod sense heritability and path analysis) were calculated using a computer software (written in basic) according the formulae by Dewey & Lu (1959) and detailed description in the book by Singh & Chaudhary (1985). In addition, the averaged data were subjected to multivariate analyses for graphic presentation both in case of cluster analysis and scattered patterns during all the three years and the STATISTICA 7.0 and SPSS 16.0 computer software were used following the procedures by Sneath & Sokal, 1973. The differences due to scale unit were standardized prior to analysis for cluster analysis, whereas the PCA were performed on the averaged data because PCA is being conducted on covariance or correlation matrices that are devoid of scaling unit.

Results

Analysis of variance, simple statistical parameters and path coefficients (direct and indirect effects) presented in the Table 1 indicated that the higher amount of variability was more attributed through year effects for days to maturity, whereas yield contributing traits were more contributed due to genotypic effects that could be exploited during the evaluation process, to avail the selection opportunity. Insignificant year effects for yield contributing traits and appropriate CV indicated the consistency of the experimental scenario that supported the wider acceptance of the results reported here. Maximum direct effect was contributed by biological yield that was followed by harvest index, whereas early maturing genotypes lowed grain yield and the only positive contribution by maturity was through indirect approach by biological yield only that highlighted the importance of biomass in genetic improvement for organic farming under rain-fed conditions.

Table 1. Analysis variance, simple statistics and path analysis of 4 traits in 77 accession of *Vigna mungo* for three years.

SOV	Year	df	Days to maturity	Biological yield (g)	Harvest index (%)	Grain yield (g)
Range	2011 *(n=77)*		56-121	2.23-59.64	7.78-91.70	0.82-23.75
	2012 *(n=77)*		60-119	2.23-50.39	4.96-41.21	0.50-16.34
	2013 *(n=77)*		55-111	2.12-45.60	8.62-53.80	0.78-14.78
	Overall *(n=77)*		57-114	2.20-47.10	8.90-55.60	0.80-15.30
Mean±SE	2011 *(n=77)*		89.66±2.21	23.42±1.35	27.93±1.57	6.13±0.48
	2012 *(n=77)*		82.09±1.89	21.89±1.16	27.70±1.09	5.66±0.37
	2013 *(n=77)*		81.79±1.77	21.57±1.03	26.49±1.07	5.61±0.35
	Overall *(n=77)*		84±1.87	22.29±1.07	27.37±1.10	5.79±0.36
	2011 *(n=77)*		420.75	603.66	681.31	286.61
Variance expressed in percent of mean	2012 *(n=77)*		335.09	470.11	328.41	184.57
	2013 *(n=77)*		293.97	381.67	332.36	168.72
	Overall *(n=77)*		304.51	394.48	343.33	175.28
MS (Year)		2	1578.39**	74.99[NS]	46.08 [NS]	6.34 [NS]
MS (Genotypes)		76	767.86**	263.78**	282.03**	30.33**
MS (Error)		152	60.52	31.42	43.64	3.57
MS (Inter-check)		9	45.21 [NS]	19.41 [NS]	24.54 [NS]	2.98 [NS]
MS (Intra-check)		19	12.34 [NS]	5.59 [NS]	9.48 [NS]	0.98 [NS]
CV %			9.20	25.14	24.13	32.59
SE			4.49	3.24	3.01	1.09
CD (0.05)			12.45	8.97	10.57	3.03
CD (0.01)			16.36	11.79	13.89	3.97
h^2 (broad sense)			79.60	71.11	64.61	71.42
Direct/indirect effect						
Days to maturity			-0.05	0.14	-0.30	r_G=-0.21
Biomass			-0.01	0.91	-0.12	r_G=0.77
Harvest index			0.03	-0.20	0.57	r_G=0.39

Based on Ward's method using Euclidean distances, four clusters were observed at on the bases of one fourth dissimilarity during 2011, three for both 2012 and 2013 (Fig. 1). During 2011, among four clusters, eleven genotypes were grouped in the cluster I, cluster II comprised of 29 genotypes, cluster III twenty seven and cluster IV consisted of 10 genotypes. Three clusters were observed on the bases of 2012 data, the cluster I consisted of 5 genotypes, cluster II forty four genotypes, whereas 28 were grouped together in the cluster III. Thirty four genotypes were grouped together in the cluster I for the year 2013, twenty nine were in cluster II and 14 genotypes were grouped in the cluster III. The check variety was in the cluster II during 2011 and 2012, whereas it was grouped in the cluster I during 2013. Short duration maturing and high yielding genotypes grouping in different clusters can be used in breeding program under rain-fed conditions also. The averaged performance for all the individual clusters during three years is presented in the Table 2. It was observed that the genotypes in the cluster II for the year 2011 were short duration in maturity and were high yielding, the genotypes in the cluster I were high yielding but medium maturing during 2012, whereas the genotypes in the cluster II were short duration but low yielding. The average performance of seventy seven accessions during 2013 revealed that fourteen genotypes in the cluster III were high yielding with average grain yield of 11.06 g plant^{-1}. It was critically observed that all the high yielding genotypes in any of the clusters produced maximum biomass, either that was transformed to the best harvest index or not.

Results based on principal components analyses (PCA) revealed four PCs for all the three years. First two PCs revealed valid contribution (Eigen value >1) amongst 77 genotypes for determining more than ⅔variability for all the three years, hence on the basis of these findings the results could be generalized for better interpretation. First two PCs that contributed 75% variability amongst all the genotypes for the year 2011, 87% for 2012 and 84% for 2013, genotypes were plotted in the scattered diagram in the Fig. 2. On the basis of 2011 results, three main groups were observed, *i.e.,* one in the lower half and two in the upper half, whereas five genotypes (19387, 19396, 19359, 19371. 45849) were away from major groups and these were distinctly diverse based on yield contributing traits and maturity. Four major groups were observed during 2012 but four genotypes (45304, 45920, 45921, 19378) were away from major groups, whereas three groups encircled all the genotypes during 2013. The distinctness of genotypes apart from any of the major group was influenced by the yearly environment fluctuations. The vectors of grain yield and biomass showed higher variability. In such layout, selection can be done through *axes*. Maturity duration exhibited low divergence toward the selection of desirable genotypes without compromising for yield potential, the extreme positions (very late maturing and very early maturing) for the genotypes would produce low yield, especially under rain-fed conditions. Harvest index with high variation but negative vector for *y-axis* would be exploited through better grain yield with appropriate biomass, *i.e.,* not very high that may lead to low productivity. Similar vector pattern was observed for the year 2012, whereas the results for the year 2013 deviated from this pattern, *i.e.,* maturity duration contributed positive to both the *axis*, biomass and grain

yield were positive for *y-axis* and negative for *x-axis*, whereas harvest index was negative for both the *axis.* It was observed that biomass exhibited positive magnitude against *y-axis* for all the three years that indicated in impartment of biomass in determining better yielding genotypes under rain-fed conditions without influence of rain duration and quantity.

In the coordination analysis both factors captured >⅔ variability and highlighted the genotypic efficiency based on studied traits. The biplots depicting genotypes in scattered against vectors (traits) through merger of two loading coordinates provided important information. Clustering indicates the similarity among the genotypes on the basis of covariance that have an indication for relatedness rather than agronomic performance. In general, genotype closer to zero are low performer, however genotype away from origin are good for overall performance on the basis of subjected traits, and can be used for crop improvement through simple selection, however the criterion should be based on the magnitude of diversity and gene-action for the trait/s of interest. Same the length of vectors represents the deviation based on pooled genotypic performance, shorter the vector length, low is the variation. The genotypes with shorter angle with vector and apart from origin are expected to produce high value of that specific trait. Genotypic performance was affected by the year effects, especially for maturity duration, however on the basis of average performance, 11 genotypes were identified and listed in the Table 3 presenting average values for the traits under consideration and standard deviation. All these genotypes produced better grain yield than check variety, whereas three genotypes (45304, 45920, 45921) were earlier than the check and produced considerably high grain yield. Overall the selected genotypes produced better biomass also that indicated the vitality of this trait for selecting suitable genotypes for rain-fed condition to have promising organic production. The selected genotypes are suggested for future use.

Discussion

Insignificant differences for the check repeated ten times indicated validity of interpretation and a non-replicated experiment (augmented design in this case) including high number of test cultivars with repeated checks make the experimentation valid. Particularly, the results based on original data are more reliable than transformed, however selection of experimental site is very important in this situation and that is pre-requisite for a experiment. Based on basic information over three years enabled us to identify better genotypes through simple selection from the germplasm that exhibited exponential variance coupled with high heritability (Jyothsna *et al.,* 2016). Variance expression in percentage hence is more practicable (Ilyas*et al.,* 2014). Singh *et al.* (2014) reported legumes' problems related to management and suggested softwares for interpretation of huge data sets. Harris & Orr (2014) explained the potential of agriculture for poverty alleviation for small farmers under rain-fed crop production in Africa and India, however crop production through increase farm size or crop diversification, commercialization and increased farm profitability was highlighted. On the other hand weed dynamics and productivity has also been discussed recently by Rai *et al.* (2016).
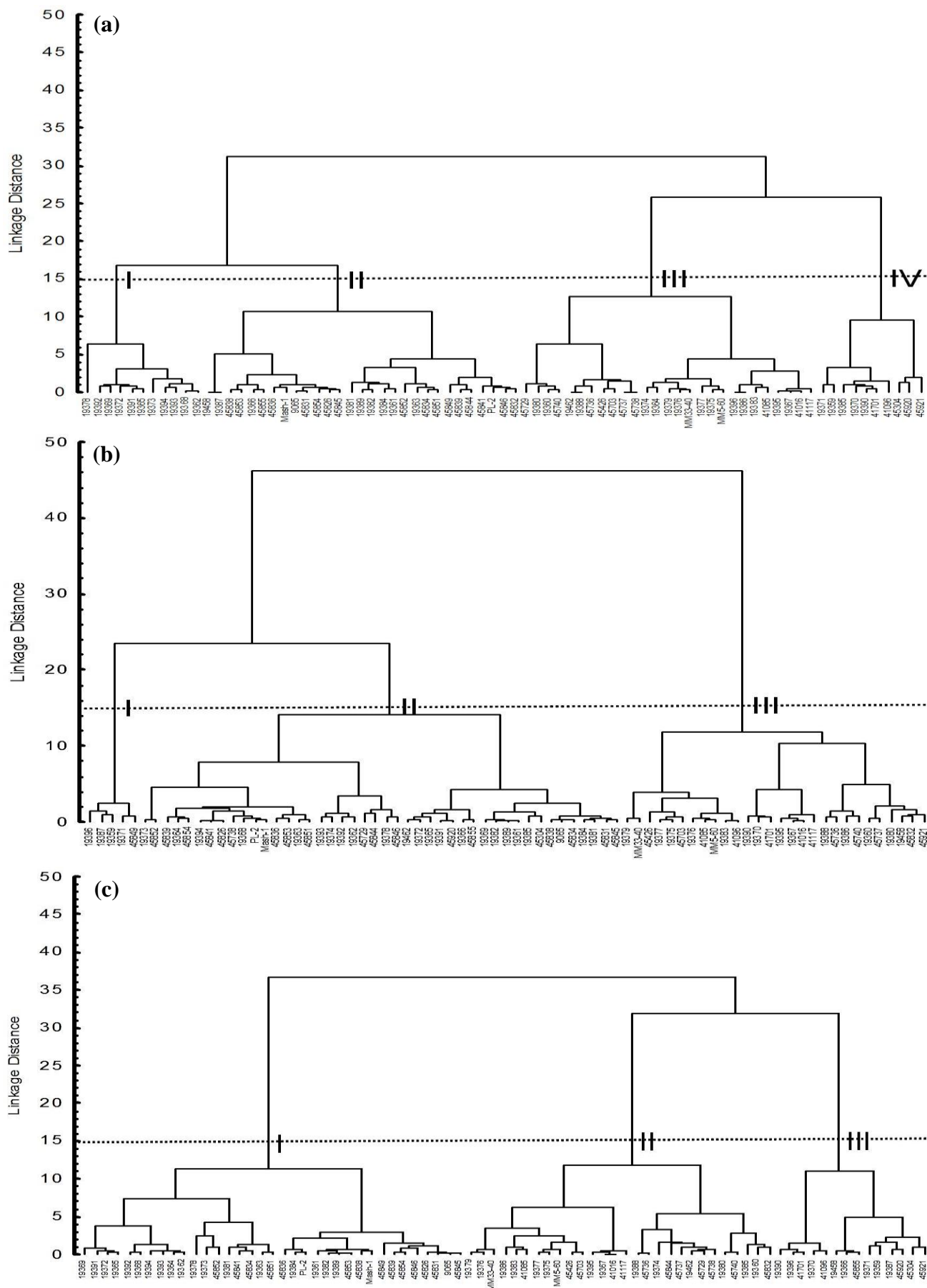
Fig. 1. Clusters for seventy seven accessions of Vigna mungo for three years evaluation under rain-fed conditions for days to maturity, biomass, grain yield and harvest index. The upper graph (a) is based on 2011, the middle (b) for 2012 and lower graph (c) is based on the data for the year 2013.

Table 2. Year-wise frequency in each cluster and cluster mean and standard deviation in 77 genotypes of
***Vigna mungo* evaluated for three years.**

Years	Cluster	Frequency	Mean ± standard deviation			
			DM	BY	GY	HI
2011	I	11	89.18±4.40	14.78±5.52	4.93±2.13	37.01±7.27
	II	29	69.90±5.86	19.17±6.94	6.10±2.33	32.82±4.81
	III	27	96.67±13.50	22.68±7.02	3.81±1.91	17.28±6.15
	IV	10	89.30±18.33	38.56±4.65	11.23±0.86	28.23±6.16
2012	I	5	80.00±16.46	34.94±6.36	11.82±2.80	33.22±5.98
	II	44	76.59±10.23	18.16±7.22	5.65±2.43	32.86±6.32
	III	28	97.89±15.04	26.53±9.08	4.95±3.24	17.71±6.60
2013	I	34	75.62±11.26	16.38±5.40	5.34±1.81	34.51±5.38
	II	29	93.97±15.05	22.33±6.74	3.79±1.70	17.59±6.10
	III	14	86.79±16.84	36.56±5.65	11.06±2.35	30.30±6.30

Table 3. Mean and standard deviation of high yielding genotypes of *Vignamungo* over three years'
average performance in comparison with the check variety.

Genotype	Days to maturity	Biological yield (g)	Grain yield (g)	Harvest index (%)
19359	81.1±12.70	42.57 ± 7.24	15.28±0.58	35.79±5.61
19366	80.7±2.46	27.55 ± 1.05	9.38±0.36	34.40±1.31
19371	87.8±17.88	35.20 ± 1.59	12.87±0.49	36.87±1.17
19387	73.4±2.15	34.35±9.66	12.70±0.49	39.59±9.56
19390	110.2±3.08	39.55±1.10	9.90±0.38	25.26±1.47
19396	101.2±16.33	37.42±7.58	10.16±6.03	25.77±12.11
19458	78.8±6.54	31.04±6.31	9.28±3.15	34.46±14.74
45304	70.3±2.03	41.62±17.04	12.38±5.54	28.74±1.55
45701	104.8±3.29	37.56±3.15	9.64±1.15	25.20±1.18
45920	66.9±2.74	34.61±11.08	12.54±4.14	35.55±1.09
45921	65.4±2.36	47.12±11.84	14.88±8.64	28.84±11.33
Mash 1	72.0±92.37	22.08±7.71	6.69±1.41	31.75±4.44

In the present investigation, high variation for yield contributing traits and maturity duration indicated the affectivity of the attribute in arid climate that would make possible to breed cultivars suitable for organic farming, especially under rain-fed agriculture. Breeding program for non-conventional paramedic combinations (organic farming and under rain-fed in present case), asystematic and precise evaluation over multiple locations and years is required for the development of suitable blackgram cultivars with stable organic cropping systems. Additional benefit of the results explained in the manuscript could be establishment of a mini core (Wei *et al.,* 2011), but additional data on molecular information is needed. However Jiang *et al.* (2014) used association mapping for exploring the molecular basis of phenotypic variations in plants in peanut mini-core and suggested model-based structure analysis assigned all accessions. More than 800 accessions of blackgram have been conserved in the genebank and among these almost 500 have been evaluated, therefore a mini core is required to be utilized intensively for the development of suitable cultivars for organic farming under rain-fed conditions. In addition, marker linkage with yellow mosaic virus resistance in black gram has recently been reported by Panigrahi *et al.* (2016).

On the basis of information, *Vigna mungo* germplasm that was indigenous possessed alleles for adaptation under organic agricultural system, and the genotypes identified under rain-fed scenario are expected to be as the base material for future blackgram development suitable for organic farming that is an emerging demand for healthcare. Jha *et al.,* (2014) suggested chickpea breeding under

climate change was expected to bring new production scenarios as the entire growing area in Indo–Pak subcontinent. In this regards reactive oxygen species (ROS) along with aluminum induction have also been studied in black gram by Chowra *et al.* (2017). Due to ecological change, warranting strategic planning for crop breeding and husbandry, the conventional breeding has produced several high-yielding chickpea genotypes without exploiting its potential yield owing to a number of constraints. The future decades are likely to see increasing pressures on the global food system, both on the demand side from increasing population and *per capita* consumption, and on the supply side from greater competition for inputs and from climate change (Singhal *et al.,* 2014).Blackgram has been originated in the Indian subcontinent (Reddy *et al.,* 2014), and the germplasm evaluated under this study was predominantly indigenous, hence potentially suitable genotypes for organic farming are expected, however Mabhaudhi & Modi (2014) emphasize the farming practice that has existed throughout history and one which mimics natural diversity. Further neglected crops too have been regarded as "traditional" crops and have been neglected by researchers, farmers and communities. Despite that they represent a rich biodiversity which has now been lost. For extensive evaluation Wani *et al.* (2015) also studied physiochemical properties in black gram. Joshi *et al.* (2014) considered mung beans another value added crop after winter filed harvest.
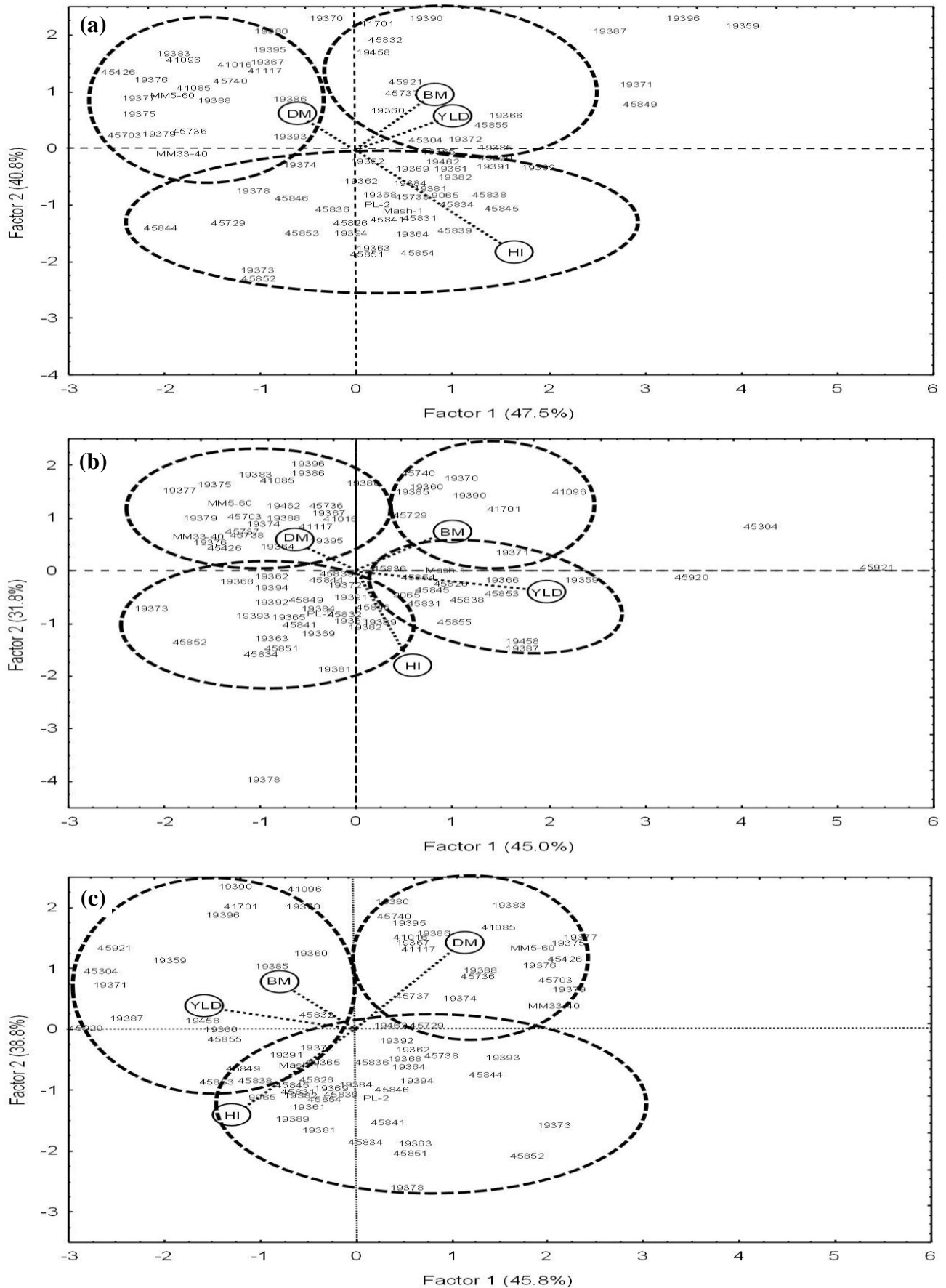
Fig. 2. Scattered diagram for seventy seven accessions of *Vigna mungo* based on first 2 factors contributing ¾ variability for three years evaluation under rainfed conditions for days to maturity, biomass, grain yield and harvest index. The upper (a) graph is based on 2011, the middle (b) for 2012 and lower graph (c) is based on the data for the year 2013. The vectors encircled are DM represents days to maturity, 3BM biomass plant-1, YLD grain yield plant-1and HI is for harvest index (%).

The challenges of organic farming could be addressed through the introduction of natural genetic variation explored among the existed resources through chemical mutagens (Bansal *et al.*, 2014). Performance of short season crops under water stress is important, especially under semi-arid regions and mungbean has been reported potential crop by Ambachew *et al.* (2014). Biomass is one of the most important selection criteria, especially for blackgram that has pivotal role for yield potential under rain-fed conditions and the genetic differences are yet to isolate for utilization. Although advances in biotechnology made crop improvement swift, but gaps between developed and underdeveloped world has limited the access to food to the poor nations, especially in the era of over-demanding for organic food that is mainly produced in the less developed world. We suggest coordinated and globalized approach to agricultural research through crop networks to produce good quality food for all (Grauwet *et al.*, 2014). In the present era of demands for organic foods, the situation has blazed breeding crops for through bio-omics that will stay closer to nature, however no single country can accomplish this task to fulfill the gap of genetic resources along with breeding for targeted traits. Keeping in view the genetic diversity of blackgram (Ghafoor *et al.*, 2012), coordination is required where is no CGIAR has blackgram demands due to altered environmental factors. Food legumes are the source of protein for the poor and at the same time increase soil fertility. In the present time molecular markers added modification in knowing the genetics of complex traits assist the breeder to detect responsible genetic regions.

To avoid the health risks, organic farming provides better measures by the application of inorganic supplements to the food crops. Without proper utilization of the genetic resources,

cost affectivity is nothing but a dream. Through widened genetic diversity (for which gene bank might be the potent source), newly incorporated genes can adapt changing eco-environment and organic farming as well. Variety with vegetable protein sources can be developed with zero inorganic inputs so that productivity may increase in arid regions as well.

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