

## GENETIC DIVERSITY IN LOCAL COLLECTIONS OF *VIGNA MUNGO* (L.) HEPPER WITH RELATION TO GEOGRAPHIC PATTERN

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### Abstract

Eighty eight genotypes of *Vigna mungo* (L.) Hepper selected on the basis of source/origin were evaluated for agronomic traits and analyzed on the basis of three geographic parameters i.e., provinces, agro-ecological zones and altitude. The differences according to geographical regions proved its validity in substantiating the postulated regions of diversity or gene centres. Migration of landraces into new regions, followed by some degree of contamination by mixture or out crossing with other landraces were observed which might be due to frequent exchange of germplasm or transportation of grains from one place to others. The areas with a high level of stress will present interesting tolerance to environmental stresses, but homogeneous mixtures, and hence needs less extensive sampling for genetic resources for conservation purposes. The study confirmed the existence of a wealth of phenotypic divergence in the local blackgram germplasm. The variation within the country appears largely attributable to different provinces rather than smaller units of crop-ecological zones and altitude intervals. Further collecting missions to main blackgram growing areas with greater diversity could concentrate efforts on sampling as many geographically and ecologically distinct areas as possible rather than collecting extensively from fields close to motorable roads within individual province. The germplasm with high mean values along with medium to high genetic variance should be exploited through simple selection.

### Introduction

One of the approaches for strengthening gene pool is to collect material from diverse geographical origins from proposed centres of diversity in individual samples. Representative samples from the complete geographical range of the crop species can help to ensure conservation of co-adapted gene complexes (Frankel *et al.*, 1995; Brown, 1978; Brown & Weir, 1983; Beuselinck & Steiner, 1992). Blackgram or mash [*Vigna mungo* (L.) Hepper] is an important summer pulse crop and widely cultivated under rainfed areas of the country. Its germplasm has been collected from various parts of the country, but more emphasis have been given to collect the material from easily approachable areas closer to the motorable roads. Although, germplasm collection from easily approachable areas is first priority due to threat of genetic erosion because advanced breeding material can adapt easily to the areas nearer to major towns (Bisht *et al.*, 1998, 1999) but to fetch maximum genetic diversity, it is important to make a comprehensive plan for expeditions to have maximum area explored, especially interior territories and valleys between high mountains where maximum genetic diversity exists. The present study was conducted to determine the genetic diversity in relation to collecting sites which were categorized into province, crop-ecological zone and altitude on the basis of agronomic traits which will ultimately help to design further collecting mission of the crop to collect maximum genetic diversity.

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**Table 1. Germplasm selected on the basis of geographic origin and evaluated for agronomic traits.**

Province/location	Number of accessions collected	Accessions planted	Accessions evaluated
Punjab	249	14	14
Sindh	7	5*	0
NWFP	35	15	15
Baluchistan	7	2	2
Azad Jammu & Kashmir	2	1	1
Northern Areas	10	10	10
Sub-total	310	47	42
Varieties/advance lines	-	30	30
Donated germplasm in the gene-bank	105	16	16
Total evaluated			88

\*The accessions collected from Sindh province could not mature at NARC, Islamabad and hence excluded from evaluation and analyses.

### Materials and Methods

Eighty eight genotypes were used as research material (Table 1) and out of these 42 were sampled on the basis of geographic origin from the country, 16 were pure-lines donated by national research stations (NRS) and 30 were advance lines along with one check variety (Mash 1) developed by Pulses Programme, National Agricultural Research Centre (NARC), Islamabad (Ghafoor, 2000). Ten plants from each accession/genotype were sampled at random during 1995 and their progenies were planted during July 1997. Two rows of 2-meter length for each plant progeny was planted with 50 and 10 cm inter and intra-row spacing, respectively. Recommended cultural practices were followed throughout the crop season to get healthy crop. Pesticides were sprayed twice to save the crop from the infestation of pests especially white fly, a vector of Mungbean Yellow Mosaic Virus (MYMV). Days to flowering were recorded when 50% plants started flowering and days to maturity were recorded at 90% maturity when pods turned brown/black. Other quantitative data i.e., branches, pods, grain yield (g) and biomass (g) were recorded on 10 competitive plants sampled randomly. Pod length (cm) and seeds per pod were recorded on 10 pods sampled at random within each accession/genotype. Pods per branch were calculated and expressed as pods per unit branch and seed weight was recorded after counting 100 seeds by seed counter and weighed in grams. Harvest index was determined as economic yield expressed in percentage over total biomass. The data were analyzed for simple statistics on the basis of geographic parameters i.e., provinces, crop-ecological zones and altitude. The crop agro-ecological zones were followed considering the physiography, climate and soils in which the country has been divided into 17 crop ecological zones (Anon., 1976).

### Results and Discussion

The means along with standard error and variance for quantitative traits presented in the Table 2 revealed that advance lines were observed earlier in flowering ( $38.1 \pm 0.58$  days) and

maturity ( $65.9 \pm 0.67$  days) whereas, the accessions from Baluchistan were late maturing ( $102.6 \pm 1.82$  days). High variance was recorded for days to maturity in accessions from Punjab (278.1), N.W.F.P. (179.1) and in donated germplasm (175.7). The accessions from Baluchistan gave the maximum variance for branches per plant (174.8) followed by advance lines (65.5). Pods per plant exhibited high genetic variation in the accessions collected from almost all the areas including the donated germplasm where the highest variance (638.8) was recorded and this may be because of diversity in collection/selection criteria. Low variance for pods was observed in the advance lines, which indicated the impact of selection pressure as the breeders select most of germplasm for high yield potential (Ghafoor *et al.*, 2000). Low variance for pod length, seeds per pod and 100-seed weight is supposed to be related to narrow genetic base as these characters are least affected by the environments. High variance was observed for biological yield, grain yield and harvest index in all accessions collected from all the locations including donated germplasm. In the germplasm donated from other institutes, high variance for most of the traits indicated the importance of this germplasm and further improvement through simple selection from this material.

The mean values and variance for agronomic traits presented in Table 3 revealed that the accessions collected from zero to 200 masl were late in maturity ( $97 \pm 2.22$  days) and gave the highest variance (295.4). It was followed by the accessions collected from 1401 to 1600 masl with an average maturity of  $82.4 \pm 2.00$  days with high variance (240.5). The accessions collected from 401 to 1400 masl were early to medium in maturity coupled with low to medium variance. The accessions from 0 to 400 masl gave high variance for maturity, pods, biological yield, grain yield and harvest index. High yielding germplasm with other good economic characters was observed in the collections from 0 to 600 masl and then from high altitude ( $> 1600$  masl). The germplasm from these sites had high grain yield along with high genetic variance, which indicated the presence of landraces in these areas and hence more collection mission may be organized to collect maximum variability for conservation and utilization. The low variance was observed for pod length, seeds per pod, seed density and seed weight, which might be related to narrow genetic base for these traits in local germplasm. Hence better germplasm particularly for these traits should be acquired from other sources to broaden the genetic base. The material collected from 1801 to 2000 masl gave fairly high mean values for 100-seed weight ( $5.14 \pm 0.13$  g) and thus may be exploited for the development of high yielding cultivars along with bold seeds.

The country has been classified in to 17 crop-ecological zones and blackgram germplasm have been collected from 10 zones, whereas others are yet to be explored. The accessions from zones 6 and 13 gave high degree of variation for days to flowering; accessions from zones 5, 6, 7 and 13 for days to maturity and accessions from zone 14 for branches (Table 4). Pods per plant were observed with high variability scattered throughout the germplasm. The accessions from zone 6 exhibited high variance for maturity, pods, biological yield, grain yield and harvest index. The accessions collected from zones 13 and 14 gave maximum variance for most of the agronomic characters and thus the germplasm from these areas should be investigated carefully for exploitation of genetic variation. The germplasm collected from zones 9 and 10 was early in maturity and low yielder with low variability for both the characters, whereas the germplasm collected from the zones 3, 4 and 14 was late in maturity but high yielder with medium to high levels of variability, therefore, the selected genotypes from these two groups of zones are suggested to be used in breeding programme to combine important plant characters in one genotype.

Table 2. Quantitative variation in accessions based on provincial distribution.

Traits	Number of samples						
	Punjab	N.W.F.P	Baluchistan	AJK	Northern Areas	Advance lines	Donated
	140	150	20	10	100	300	160
Days to flowering	46.8±0.71	43.9±0.73	52.4±1.19	48.0±0.26	49.77±1.08	38.1±0.58	47.3±0.61
	70.9	79.9	28.15	0.67	104.9	10.2	64.2
Days to maturity	84.0±1.41	78.6±1.09	102.6±1.82	95.3±0.26	92.74±1.38	65.9±0.67	90.4±1.02
	278.1	179.1	66.04	0.68	171.6	13.3	175.7
Branches/plant	20.1±0.66	12.3±0.50	23.08±2.96	12.6±1.80	15.69±0.72	18.9±1.48	15.79±0.57
	60.3	37.0	174.8	32.27	46.11	65.5	55.5
Pods/plant	37.3±1.33	25.8±1.64	43.18±4.76	27.4±4.49	35.94±1.90	37.0±1.88	33.1±1.94
	246.4	404.4	452.3	202.04	323.7	106.5	638.8
Pods/branch	1.93±0.05	2.02±0.08	2.12±0.20	2.27±0.33	2.31±0.08	2.23±0.12	2.12±0.08
	0.34	0.92	0.81	1.11	0.58	0.40	1.18
Pod length	4.53±0.03	4.28±0.02	4.61±0.06	4.92±0.11	4.67±0.03	4.38±0.03	4.53±0.03
	0.13	0.08	0.08	0.13	0.07	0.03	0.18
Seeds/pod	6.20±0.04	5.90±0.05	6.65±0.09	6.26±0.17	6.32±0.06	6.01±0.04	6.14±0.05
	0.17	0.32	0.15	0.28	0.35	0.06	0.50
100-seed weight	4.41±0.06	3.86±0.07	4.45±0.08	5.11±0.16	4.5±0.07	4.23±0.06	4.44±0.04
	0.46	0.78	0.12	0.25	0.39	0.12	0.26
Biomass (g)	32.47±1.52	18.19±1.18	36.40±3.81	28.35±4.66	29.39±1.58	23.98±1.68	24.72±1.17
	323.87	208.4	290.26	217.5	225.0	85.01	230.9
Grain yield (g)	6.14±0.30	4.52±0.42	10.05±1.05	5.95±1.18	7.48±0.43	6.57±0.49	6.71±0.47
	12.89	26.85	21.99	13.95	16.86	7.32	37.38
Harvest index	20.12±0.69	21.68±0.74	28.24±1.00	20.87±2.34	26.14±0.74	27.58±0.81	26.08±0.84
	66.34	81.72	19.9	54.95	49.47	19.63	120.9

Upper row presents mean±SE and lower row is variance for specific character.

Table 3. Quantitative variation in accessions based on crop-ecological regions.

Traits	Number of samples									
	3	4	5	6	7	9	10	12	13	14
	10	10	20	20	30	40	40	60	170	20
Days to flowering	57.5±0.17	63.2±0.25	53.1±0.56	55.2±3.04	49.2±1.42	39.7±0.20	40.6±0.12	41.2±0.43	46.52±0.76	52.4±1.19
	0.28	0.62	6.31	185.3	60.3	1.60	0.61	11.2	99.41	28.15
Days to maturity	110.5±0.17	107.4±0.16	98.7±2.72	95.2±3.50	88.3±2.54	70.5±0.65	70.7±0.29	76.2±1.17	86.1±1.14	102.6±1.82
	0.28	0.27	148.5	245.1	193.3	16.97	3.45	82.8	221.47	66.04
Branches/plant	35.7±0.78	24.8±1.46	19.9±1.26	12.5±1.34	21.6±1.38	14.93±0.85	16.58±1.03	9.2±0.61	15.3±0.49	23.1±2.96
	6.05	21.41	31.74	36.09	57.5	29.1	42.56	22.1	40.61	174.83
Pods/plant	60.2±3.94	56.0±4.06	26.6±2.62	25.6±3.25	39.6±2.91	30.0±1.73	31.0±2.49	15.9±1.49	35.1±1.52	43.2±4.76
	155.6	164.94	137.26	210.9	253.7	119.2	247.6	133.2	392.95	452.34
Pods/branch	1.67±0.07	2.27±0.11	1.39±0.14	2.03±0.14	1.91±0.09	2.10±0.10	1.85±0.11	1.74±0.10	2.29±0.07	2.12±0.20
	0.05	0.13	0.41	0.40	0.27	0.41	0.47	0.61	0.82	0.81
Pod length	4.45±0.06	5.04±0.05	5.14±0.09	6.64±0.08	4.31±0.02	4.39±0.02	4.30±0.04	4.26±0.05	4.50±0.02	4.61±0.06
	0.04	0.02	0.18	0.13	0.02	0.02	0.06	0.16	0.08	0.08
Seeds/pod	6.50±0.03	6.52±0.07	6.44±0.11	6.04±0.18	6.09±0.09	6.21±0.06	5.73±0.08	5.91±0.07	6.15±0.04	6.65±0.09
	0.01	0.05	0.23	0.61	0.23	0.12	0.26	0.29	0.31	0.15
100-seed weight	4.29±0.09	4.68±0.09	5.38±0.11	4.60±0.25	4.32±0.16	4.14±0.06	3.84±0.12	3.51±0.11	4.40±0.05	4.45±0.08
	0.08	0.09	0.26	1.25	0.75	0.14	0.55	0.68	0.38	0.12
Biomass (g)	48.02±3.86	53.0±1.85	30.72±2.01	27.56±5.05	46.01±4.46	19.01±1.10	22.88±1.44	12.58±1.44	25.50±1.12	36.40±3.81
	149.21	34.34	80.79	509.45	596.5	48.58	82.57	24.14	211.82	290.26
Grain yield (g)	13.13±1.17	10.29±0.83	5.19±0.54	5.99±1.15	5.27±0.58	5.01±0.42	4.26±0.46	2.56±0.34	6.73±0.39	10.05±1.05
	13.78	6.94	5.90	26.61	10.27	6.99	8.30	6.98	25.82	21.99
Harvest index	27.15±0.26	19.19±1.15	16.64±1.64	23.99±1.67	11.53±0.80	25.82±0.82	17.57±1.55	20.30±0.80	24.60±0.65	28.24±1.00
	0.68	13.12	53.85	56.09	19.17	33.6	96.29	38.88	72.29	19.90

Upper row presents mean±SE and lower row is variance for specific character.

Table 4. Quantitative variation in accessions based on altitude.

Traits	Number of samples											
	> 200	> 400	> 600	> 800	> 1000	> 1200	> 1400	> 1600	> 1800	> 2000	< 2200	2400
Days to flowering	51.7±1.23	47.7±1.18	40.4±0.11	38.9±0.35	40.6±0.11	44.9±1.47	39.2±0.22	44.9±1.47	51.2±0.90	54.6±1.98	43.0±1.44	51.2±0.51
	60.60	84.1	0.25	3.8	0.26	108.6	0.96	129.2	56.9	78.5	41.7	2.6
Days to maturity	97.0±2.72	84.5±1.89	68.9±0.37	71.5±0.30	73.9±0.81	77.4±1.80	68.7±0.32	82.4±2.00	96.0±1.52	101.1±1.32	88.8±1.95	96.2±0.29
	295.4	214.0	2.8	2.7	13.2	161.9	2.01	240.5	162.3	34.73	75.7	0.84
Branches/plant	23.8±1.51	19.1±0.91	15.7±0.68	15.8±1.21	16.9±1.82	10.0±0.83	10.9±0.41	12.1±0.63	18.7±1.16	15.7±1.28	15.5±1.29	16.6±2.73
	91.4	49.9	9.38	43.6	66.1	34.31	3.29	23.8	94.5	32.8	33.5	74.27
Pods/plant	40.6±3.15	36.8±1.94	30.7±1.54	32.5±2.49	26.6±3.38	17.0±1.85	18.6±1.27	25.7±2.49	41.1±2.33	39.5±4.67	35.3±3.65	51.6±9.29
	396.8	226.2	47.4	186.7	227.9	170.42	32.5	373.0	378.6	436.4	1266.4	862.9
Pods/Branch	1.77±0.09	2.02±0.09	1.98±0.08	2.12±0.13	1.57±0.11	1.71±0.10	1.66±0.06	2.05±0.13	2.36±0.11	2.44±0.13	2.28±0.15	3.21±0.46
	0.34	0.44	0.14	0.48	0.24	0.45	0.08	1.03	0.81	0.35	0.46	2.11
Pod length	4.64±0.08	4.55±0.04	4.43±0.02	4.41±0.03	4.23±0.03	4.15±0.04	4.16±0.04	4.39±0.05	4.69±0.03	4.71±0.04	4.66±0.07	4.58±0.08
	0.27	0.10	0.01	0.02	0.02	0.07	0.04	0.12	0.07	0.04	0.09	0.06
Seeds/pod	6.32±0.07	6.22±0.06	6.20±0.06	5.90±0.05	5.91±0.06	5.86±0.08	5.73±0.12	5.85±0.09	6.44±0.06	6.50±0.11	6.26±0.11	6.20±0.19
	0.18	0.20	0.08	0.08	0.07	0.34	0.27	0.54	0.23	0.26	0.23	0.35
100-seed weight	4.47±0.15	4.54±0.07	4.14±0.05	4.10±0.13	3.86±0.14	3.54±0.12	3.56±0.09	4.10±0.12	4.57±0.05	5.14±0.13	4.31±0.07	4.82±0.14
	0.93	0.29	0.0	0.48	0.39	0.70	0.18	0.85	0.19	0.32	0.11	0.19
Biomass (g)	38.31±3.30	34.23±2.44	25.26±1.79	18.57±1.48	19.34±2.24	12.87±1.64	9.82±0.83	21.44±2.07	33.38±1.68	34.19±3.64	25.82±2.67	35.03±6.45
	435.92	356.0	64.22	65.90	100.65	134.21	13.86	256.36	198.06	264.28	142.65	416.32
Grain yield (g)	6.49±0.73	6.60±0.45	5.79±0.51	4.20±0.41	3.41±0.45	2.76±0.42	4.68±0.13	4.81±0.68	8.78±0.51	9.30±1.31	6.95±0.65	11.98±2.35
	21.15	12.40	5.11	4.94	4.00	8.83	0.35	27.63	18.45	34.12	8.37	55.42
Harvest index	17.06±1.28	20.98±1.07	23.13±1.10	22.69±1.42	17.97±1.01	21.06±0.90	17.68±0.80	20.04±1.47	26.45±0.79	25.88±1.49	27.94±1.39	34.06±3.12
	65.47	69.22	24.06	60.72	20.31	40.55	12.72	128.94	44.12	44.28	38.79	97.61

Upper row presents mean±SE and lower row is variance for specific character.

### **Maturity influenced by geographical parameters**

The mean values and variance for maturity based on provinces revealed that advance lines has no further potential for selecting short duration cultivars, because these lines matured up to 66 days after planting and no genetic variation was observed. The accessions from NWFP and Punjab revealed a good scope for selection. On the other hand, material collected from Azad Jammu & Kashmir (AJK), Baluchistan and Northern Areas (NA) was late maturing and exhibited low to medium variance (Fig. 1). On the basis of altitude, the results revealed that the accessions collected from 401 to 1400 masl were in general early maturing but due to low variance except for 1001 to 1200 masl, further improvement for earliness is limited. Accessions collected from 0 to 400 masl and 1401 to 1800 masl though were late in maturity but exhibited high genetic variance and thus indicated the scope for further selection (Fig. 2). Crossing among selected parents from these identified groups may produce desirable recombinants for further selection. The graphic presentation of mean values and variance on the basis of crop-ecological zones indicated that accessions from zones 9 and 10 were short duration with the lowest variability (Fig. 3). The accessions from zones 5, 6, 7 and 13 were late in maturity with high genetic variability that could be exploited through simple selection.

### **Grain yield influenced by geographical parameters**

The germplasm collected from Baluchistan gave the highest mean yield but this may be misleading because of small sample size and hence further collection from this area is suggested to find the accurate indication for yield potential and genetic variation. Anyhow, this material need further testing under a wide range of agro-ecological climate to confirm adaptability. Although germplasm collected from NWFP, gave high variance but the average grain yield was minimum and hence the selection for further improvement may not increase yield potential (Fig. 4). The donated germplasm exhibited high values for mean yield along with high variance and hence simple selection can be practiced for yield potential improvement in blackgram. The germplasm collected from 0 to 400 masl gave medium yield along with medium variance, whereas germplasm collected from 1601 to 2400 masl exhibited medium to high mean grain yield along with medium to high degree of genetic variation (Fig. 5). This revealed the scope of further collection and selection to build a gene pool with desirable variability for exploitation in blackgram improvement programme. The graphic presentation for grain yield in Fig. 6 revealed that the accessions collected from zone 3 gave the highest mean grain yield with medium variability and thus high yielding blackgram cultivars can be selected from this set of material. The germplasm collected from the zone 6, 13 and 14 was observed promising especially from zone 14 where high mean value along with high genetic variation revealed that careful selection should be practiced for improvement of yield potential in blackgram.

In advance breeding lines, early maturity and low variance indicated the influence of selection pressure on blackgram germplasm as modern blackgram cultivars are being selected on the basis of earliness and high yield potential to be incorporated in various cropping patterns. High variance for maturity in accessions from Punjab (278.1), N.W.F.P (179.1), NA (171.6) and in donated germplasm (175.7) indicated that considerable genetic

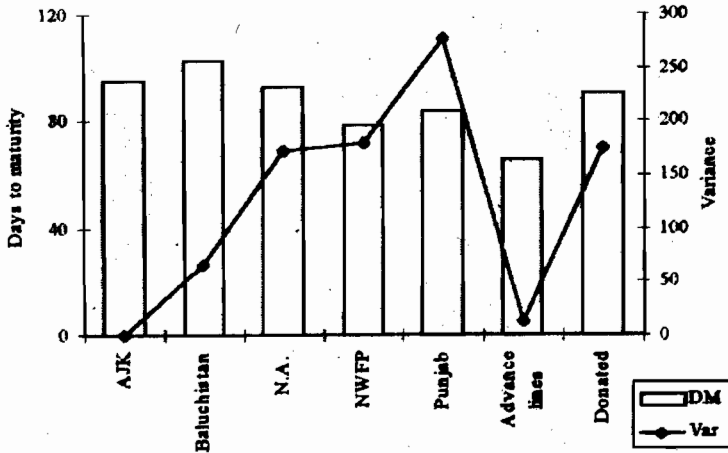


Fig. 1. Mean and variance for days to maturity on the basis of provinces in blackgram germplasm.

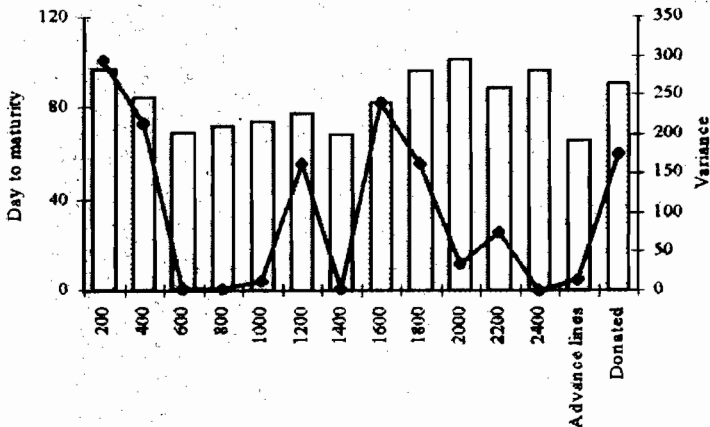


Fig. 2. Mean and variance for days to maturity on the basis of altitude of blackgram germplasm.

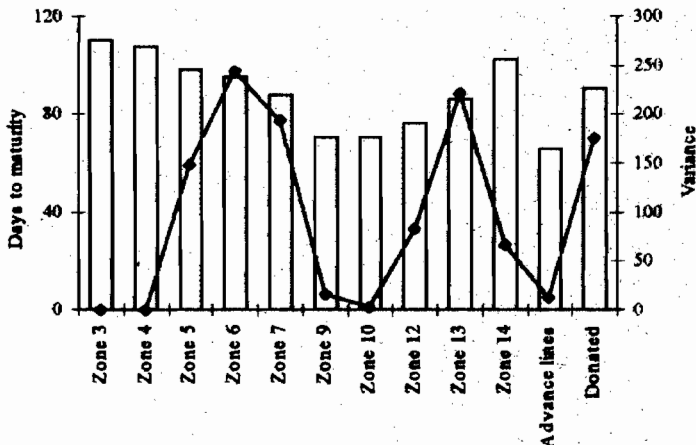


Fig. 3. Mean and variance for days to maturity on the basis of crop-ecological zones in blackgram germplasm.



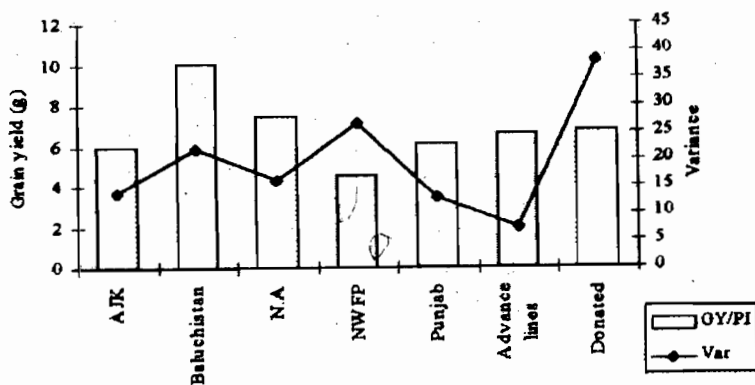


Fig. 4. Mean and variance for grain yield (g) on the basis of provinces in blackgram germplasm.

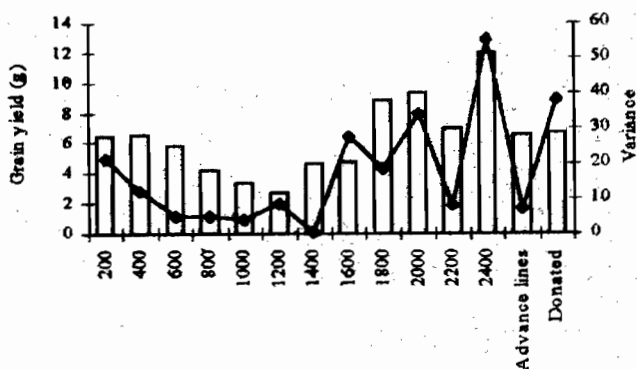


Fig. 5. Mean and variance for grain yield (g) on the basis of altitude in blackgram germplasm.

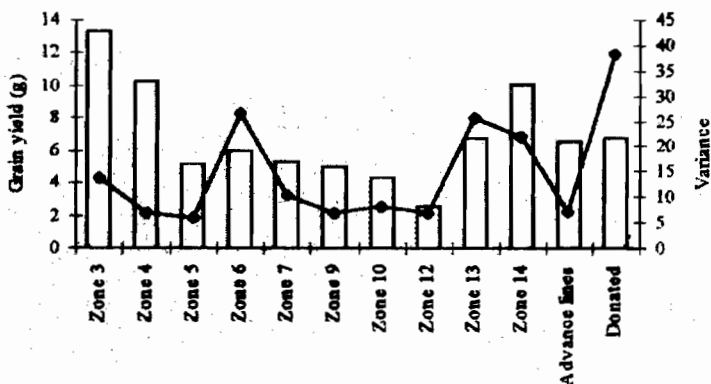


Fig. 6. Mean and variance for grain yield (g) on the basis of crop-ecological zones in blackgram germplasm.

variability for maturity is present in these areas which could be collected and utilized for developing high yielding cultivars for various maturity groups suitable for different cropping patterns. The germplasm collected from Baluchistan gave the highest average yield but matured very late, whereas advance lines and the accessions collected from N.W.F.P. produced medium to low grain yield and hence selected parents from these identified diverse origin are suggested to be used in breeding programme. The differences according to geographical regions shown by the analysis of quantitative traits is useful in substantiating the postulated regions of diversity or gene centres (Smith *et al.*, 1991, 1995). Genetic diversity in the present study indicated the worth in examining the centre of genetic diversity in blackgram. The rare alleles, each only occurring in one or two apparently random populations can be considered to be mutants, migration or the results of other coincidental events (Van Hintum & Elings, 1991). Alleles common in the restricted areas occur mostly in high mountainous areas. This could indicate that genetic material is introduced from foothills of NWFP to high mountains of NWFP and NA. Migration of landraces into new regions, followed by some degree of contamination by mixture or out crossing with other landraces can be expected in the country like Pakistan, where germplasm movement is not restricted from one area to other. The areas with a high level of stress are expected to present interesting tolerance to environmental stresses, but occurring in homogeneous mixtures, and hence, need less extensive sampling for genetic resources conservation purposes. The germplasm collected from areas of environmental stresses could provide a base material for developing cultivars tolerant to environmental stresses. Laghetti *et al.*, (1998) considered oppressive prolonged drought a serious threat to the conservation of gene pool of *Vigna savi* in natural habitat and thus recommended germplasm collection mission for conservation of maximum genetic diversity from the areas under environmental stresses. The areas of Baluchistan, high mountains of NWFP and NA along with AJK need to be exploited before the spread of advanced breeding lines, whereas the areas of Punjab need less attention at this stage because of the adaptation of improved varieties. The germplasm collected from the province of Sindh need special care for characterization and evaluation because of specific environmental conditions of the collecting sites. This material is expected to provide some good lines tolerant to drought.

The study confirmed the existence of a wealth of phenotypic divergence in the local blackgram germplasm, hence support the hypothesis that this part of world might be the centre of diversity for blackgram as already considered by Vavilov (1951). The variation within the country appears largely attributable to different provinces, therefore for investigating genetic diversity in relation to geographic patterns, large units of collection sites should be considered for comprehensive discussions. Further collecting missions to main blackgram growing areas with greater diversity could concentrate efforts on sampling as many geographically and ecologically distinct areas as possible, rather than collecting extensively from fields close to motorable roads within individual province as already has been suggested by Pecetti *et al.*, (1992, 1996) for tetraploid wheat. Laghetti *et al.*, (1998) suggested collecting expedition to the areas where genetic erosion takes place in cowpea along with the areas where existing genetic diversity has not been yet gathered (Padulosi, 1993).

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