

**VARIABILITY STUDIES IN M₃ GENERATION AND
SCREENING FOR YELLOW MOSAIC VIRUS DISEASE
RESISTANCE IN ISOLATED MUTANTS OF BAMBARA
GROUNDNUT [*Vigna subterranea* (L.) Verdc.]**

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G.K.V.K. CAMPUS, BENGALURU – 560065**

2015

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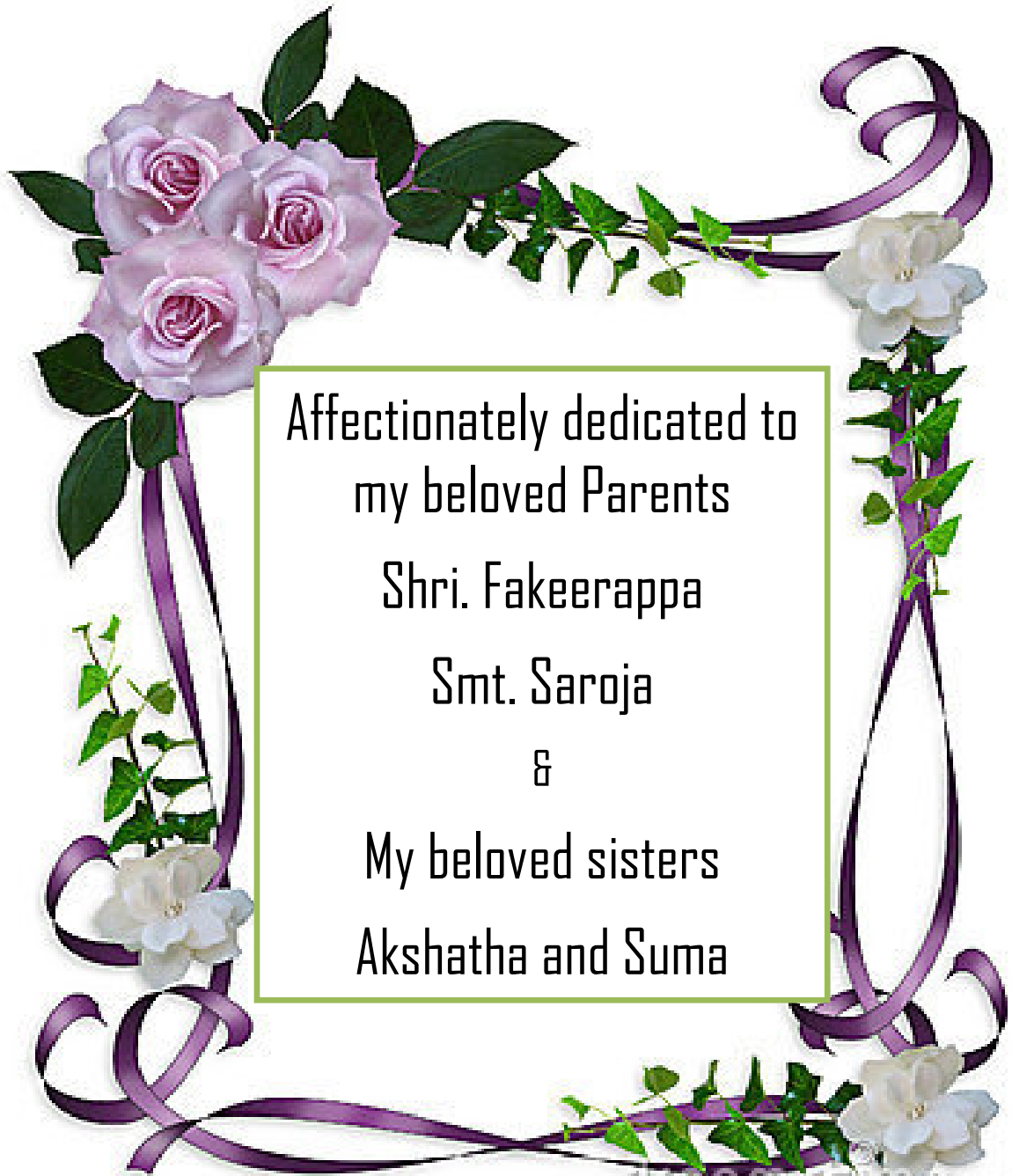
**PRANESH
PALB 3215**

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In partial fulfilment of the requirements
for the award of the degree of

Master of Science (Agriculture)
in
GENETICS AND PLANT BREEDING

BENGALURU

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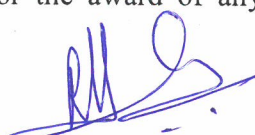


**DEPARTMENT OF GENETICS AND PLANT BREEDING
COLLEGE OF AGRICULTURE, GKV, BENGALURU
UNIVERSITY OF AGRICULTURAL SCIENCES, BENGALURU**

CERTIFICATE

This is to certify that the thesis entitled “**VARIABILITY STUDIES IN M₃ GENERATION AND SCREENING FOR YELLOW MOSAIC VIRUS DISEASE RESISTANCE IN ISOLATED MUTANTS OF BAMBARA GROUNDNUT [*Vigna subterranea* (L.) Verdc.]**” submitted by **Mr. PRANESH** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **GENETICS AND PLANT BREEDING** to the University of Agricultural Sciences, Bengaluru, is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar titles.

**Bengaluru
July, 2015**


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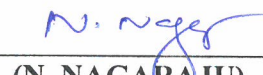
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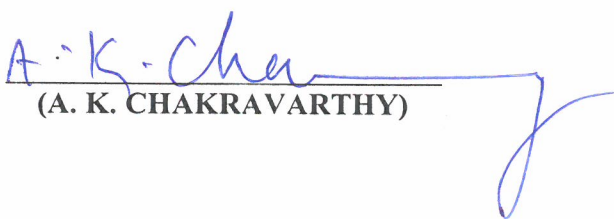
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(PRANESH)

Variability studies in M₃ generation and screening for yellow mosaic virus disease resistance in isolated mutants of Bambara groundnut (*Vigna subterranea* (L.) Verdc.)

PRANESH

ABSTRACT

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an autogamous legume crop cultivated by the resource poor farmers in the African continent. The present investigations were undertaken to study the Genetic variability, to determine the correlation and path coefficient in M₃ generation of Bambara groundnut variety 'SB-42' treated with Ethyl Methane Sulphonate and screening for yellow mosaic virus (YMV) disease resistance using heterologous probes in M₆ mutant lines irradiated with gamma rays was also undertaken during *kharif* 2014 in Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru.

In M₃ generation a positive shift in mean was observed for all traits except days to flowering and plant height. Number of branches plant⁻¹ showed high phenotypic coefficient of variance (24.53) and genotypic coefficient of variance (24.22). Higher heritability coupled with high genetic advance percent of mean was observed for the number of branches plant⁻¹, plant spread, number of pods plant⁻¹, pod yield plant⁻¹, seed yield plant⁻¹ reveals the higher genetic gain under selection. The pod yield plant⁻¹ (0.94**), number of pods plant⁻¹ and plant height had significant correlation with seed yield plant⁻¹. Pod yield plant⁻¹ (1.05), shelling percentage, number of pods plant⁻¹, plant spread, 100 seed weight and plant dry weight had higher positive direct effect on seed yield plant⁻¹. The designed SSR primers for the RGA 1F-CG/RGA 1R sequence were unable to amplify M₆ mutant lines indicating that all the lines lack resistant source and to use more markers to identify the resistance to YMV disease in Bambara groundnut.

July, 2015

Department of Genetics and Plant Breeding
UAS, GKVK, Bengaluru-65

R. Nandini
(Major Advisor)

**ಬಂಬಾರಾ ಶೇಂಗಾ (ವಿಗ್ನಾ ಸಬ್ಬೆರೇನಿಯಾ (ಎಲ್.) ವಿರಿಡಿಕ್.) ಬೆಳೆಯ ಎಮ್ಮೆ ಪೀಳಿಗೆಯಲ್ಲಿ
ವೈವಿಧ್ಯತೆ ಅಧ್ಯಯನ ಮತ್ತು ಪ್ರತ್ಯೇಕಿಸಲ್ಪಟ್ಟ ಉತ್ಪರಿವರ್ತಕಗಳಲ್ಲಿ ಹಳದಿ ಮಚ್ಚೆ
ನಂಜುರೋಗ ನಿರೋಧಕದ ಪರಿಶೀಲನೆ.**

ಪ್ರಾಣೇಶ

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಬಂಬಾರಾ ಶೇಂಗಾ (ವಿಗ್ನಾ ಸಬ್ಬೆರೇನಿಯಾ (ಎಲ್.) ವಿರಿಡಿಕ್.)ವು ಸ್ವಕೀಯ ಪರಾಗ ಸ್ಪರ್ಶದಿಂದ ಸಂತಾನೋಭಿವೃದ್ಧಿಯನ್ನು ಹೊಂದುವ ದ್ವಿದಳ ಧಾನ್ಯ ಬೆಳೆಯಾಗಿದ್ದು ಹೆಚ್ಚಾಗಿ ಆಫ್ರಿಕಾ ಖಂಡದಲ್ಲಿ ಬೆಳೆಯಲಾಗುತ್ತಿದೆ. ಪ್ರಸ್ತುತ ಅಧ್ಯಯನವನ್ನು ಬಂಬಾರಾ ಶೇಂಗಾದ ಎಸ್ ಬಿ - ೪೨ ತಳಿಯನ್ನು ಈಥೈಲ್ ಮೀಥೇನ್ ಸಲ್ಫೋನೇಟ್ ನಿಂದ ಉಪಚರಿಸಿದ ಎಮ್ಮೆ ಪೀಳಿಗೆಯಲ್ಲಿ ವಂಶವಾಹಿ ವೈವಿಧ್ಯತೆ, ಗುಣಗಳ ಸಹಸಂಬಂಧ ಮತ್ತು ಪಥ ಗುಣಾಂಕ ತಿಳಿಯಲು ಮತ್ತು ಗಾಮಾ ವಿಕಿರಣದಿಂದ ಉಪಚರಿಸಿದ ಎಮ್ಮೆ ಉತ್ಪರಿವರ್ತಕಗಳಲ್ಲಿ ಹಳದಿ ಮಚ್ಚೆ ನಂಜುರೋಗ ನಿರೋಧಕದ ಪರಿಶೀಲನೆಯನ್ನು ಹೆಟೆರೋಲೋಗಸ್ ಪ್ರೋಬ್ಸ್ ಬಳಕೆಯಿಂದ ತಿಳಿಯಲು ೨೦೧೪ನೇ ಇಸವಿಯ ಮುಂಗಾರು ಋತುವಿನಲ್ಲಿ ಅನುವಂಶೀಯತೆ ಮತ್ತು ಸಸ್ಯತಳಿ ಅಭಿವೃದ್ಧಿ ಶಾಸ್ತ್ರ ವಿಭಾಗ, ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ, ಜಿಕೆವಿಕೆ, ಬೆಂಗಳೂರು, ಇಲ್ಲಿ ಮೌಲ್ಯಮಾಪನವನ್ನು ಮಾಡಲಾಯಿತು.

ಎಮ್ಮೆ ಪೀಳಿಗೆಯಲ್ಲಿ ಹೂಬಿಡಲು ಬೇಕಾಗುವ ದಿನಗಳು ಮತ್ತು ಸಸ್ಯದ ಎತ್ತರ ಗುಣಲಕ್ಷಣಗಳನ್ನು ಹೊರತುಪಡಿಸಿ ಇನ್ನುಳಿದ ಎಲ್ಲಾ ಗುಣಲಕ್ಷಣಗಳ ಸರಾಸರಿ ಕಾರ್ಯಕ್ಷಮತೆಯಲ್ಲಿ ಧನಾತ್ಮಕ ಕದಲಿಕೆ ಕಂಡುಬಂದಿದೆ. ರೆಂಬೆಗಳ ಸಂಖ್ಯೆ ಗುಣವು ಹೆಚ್ಚು ದೃಶ್ಯವಾಹಿ ರೂಪಗುಣಾಂಕ ವಿಭಿನ್ನತೆ (೨೪.೫೩) ಮತ್ತು ವಂಶವಾಹಿ ರೂಪಗುಣಾಂಕ ವಿಭಿನ್ನತೆಯನ್ನು (೨೪.೨೨) ಹೊಂದಿದೆ. ಹೆಚ್ಚು ಅನುವಂಶೀಯತೆಯ ಜೊತೆ ಜೊತೆಗೆ ಹೆಚ್ಚಿನ ಅನುವಂಶೀಯತೆಯ ಪ್ರಗತಿ ಮುಂಚಿತವು ರೆಂಬೆಗಳ ಸಂಖ್ಯೆ, ಸಸ್ಯದ ಹರಡುವಿಕೆ, ಕಾಯಿಗಳ ಸಂಖ್ಯೆ, ಕಾಯಿಯ ಇಳುವರಿ ಮತ್ತು ಕಾಳಿನ ಇಳುವರಿ ಗುಣಲಕ್ಷಣಗಳಲ್ಲಿ ಕಂಡುಬಂದಿದ್ದು ಇವುಗಳು ಸಸ್ಯದ ಆಯ್ಕೆಯಲ್ಲಿ ಪ್ರಮುಖ ಪಾತ್ರ ವಹಿಸುತ್ತವೆ. ಕಾಳಿನ ಇಳುವರಿಯ ಜೊತೆಗೆ ಕಾಯಿಯ ಇಳುವರಿ (೦.೯೪**), ಕಾಯಿಗಳ ಸಂಖ್ಯೆ ಮತ್ತು ಸಸ್ಯದ ಎತ್ತರ ಗುಣಲಕ್ಷಣಗಳು ಹೆಚ್ಚು ಮಹತ್ವಪೂರ್ಣ ಸಹಸಂಬಂಧ ಹೊಂದಿವೆ. ಕಾಯಿಯ ಇಳುವರಿ (೦.೦೫), ಸಿಪ್ಪೆ ಸುಲಿವಿಕೆಯ ಪ್ರತಿಶತ, ಕಾಯಿಗಳ ಸಂಖ್ಯೆ, ಸಸ್ಯದ ಹರಡುವಿಕೆ, ನೂರು ಕಾಳಿನ ತೂಕ ಮತ್ತು ಸಸ್ಯದ ಒಣಗಿದ ತೂಕವು ಕಾಳಿನ ಇಳುವರಿಯ ಜೊತೆಗೆ ಧನಾತ್ಮಕ ಮತ್ತು ನೇರ ಸಂಬಂಧವನ್ನು ಹೊಂದಿವೆ ಮತ್ತು ಇವುಗಳು ಸಸ್ಯದ ಆಯ್ಕೆಯಲ್ಲಿ ಪ್ರಮುಖ ಪಾತ್ರ ವಹಿಸುತ್ತವೆ ಎಂದು ಪಥಗುಣಾಂಕದಿಂದ ತಿಳಿದುಬಂದಿದೆ. ಆರ್ ಜಿ ಎ ೧ಎಫ್ - ಸಿಜಿ/ ಆರ್ ಜಿ ಎ ೧ ಆರ್ ಆವರ್ತನದಿಂದ ರಚಿಸಿದ ಎಸ್ಎಸ್ಆರ್ ನಮೂದುಗಳು ಹಳದಿ ಮಚ್ಚೆ ನಂಜುರೋಗ ನಿರೋಧಕವನ್ನು ಎಮ್ಮೆ ಉತ್ಪರಿವರ್ತಕಗಳಲ್ಲಿ ದೃಢಪಡಿಸಲು ವಿಫಲಗೊಂಡಿವೆ. ಆದ್ದರಿಂದ ಈ ಉತ್ಪರಿವರ್ತಕಗಳಲ್ಲಿ ರೋಗ ನಿರೋಧಕ ಆಕರದ ಕೊರತೆ ಇದೆ ಮತ್ತು ಹೆಚ್ಚಿನ ಸಂಖ್ಯೆಯ ಗುರುತುಗಳನ್ನು ಬಳಕೆಮಾಡಬೇಕೆಂದು ತೀರ್ಮಾನಿಸಲಾಗಿದೆ.

ಜುಲೈ, ೨೦೧೫

ಅನುವಂಶೀಯತೆ ಮತ್ತು ಸಸ್ಯತಳಿ ಅಭಿವೃದ್ಧಿ ವಿಭಾಗ

ಯು ಎ ಎಸ್, ಜಿ ಕೆ ವಿ ಕೆ, ಬೆಂಗಳೂರು - ೬೫

ಆರ್. ನಂದಿನಿ

(ಮುಖ್ಯ ಸಲಹೆಗಾರರು)



Genetic variability studies on M₃ generation of Bambara groundnut (*Vigna subterranea* (L.) Verdc).

Pranesh and R Nandini

Department of Genetics and Plant Breeding (GPB)

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Introduction

- Bambara groundnut (*Vigna subterranea* (L.) verde.) with (2n=22) is a grain legume crop and belongs to family Fabaceae. It is grown for grain purpose mainly in African continent.
- Creation of genetic variability is the pre requisite activity in any of the breeding programmes. Variability can be created either conventionally through hybridization programmes or non conventionally by treating with mutagens.
- The major advantage in induced mutations is that multiple trait mutants can be isolated as compared to the other breeding methods where single trait can be introduced in the crop.

Objective:

- Estimation of variability parameters of different biometric traits in M₃ generation.

Material and methods

- The material for the study consisted of M₃ population derived from better performing M₂ families of SB-42 (cultivated variety).
- The M₃ population was grown in *kharif* 2014 at K block experimental plots of the Department of Genetics and Plant breeding, GKVK, University of Agricultural Sciences, Bengaluru.
- All recommended package of practices along with crop protection measures have been taken up to raise healthy plants.
- All the plants of progeny row as well as parents were used to measure pod yield and its component traits in M₃ generation

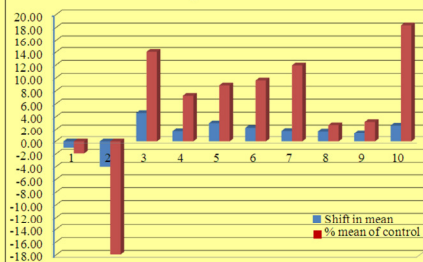
Observation recorded:

The observations on the 10 quantitative traits were recorded for the all plants of M₃ progenies

Statistical Analysis:

- The impact of mutation on M₃ generation was assessed by comparing mean, shift in mean and percent increase of mutant mean over the parent mean was calculated and depicted in Table 1.
- Genetic parameters like range, phenotypic coefficient of variance, genotypic coefficient of variance, heritability and genetic advance percent of mean was calculated and depicted in Table 2.

Figure 1: Effect of mutation on pod yield and its component traits



Experimental results

- The mutants exhibited higher mean performance compared to parents for all the traits except days to flowering and plant height (Table 1).
- With reference to shift in mean, plant height found with highest shift towards negative direction (-17.89) where as plant dry weight showed highest shift towards positive direction (18.38) was observed compare to shift in mean of all other studied traits (Table 2).
- Moderate PCV coupled with moderate GCV has been observed for all the traits except branches per plant (24.53 & 24.22) where it was noticed with higher PCV and higher GCV and for days to flowering, shelling percentage (5.32 & 4.44) and 100 seed weight (8.70 & 7.65) had with low PCV and Low GCV (table 2).
- Higher heritability along with high GAM was observed for branches per plant (97.52 & 49.28), plant height, pods per plant, pod yield per plant and seed yield per plant. Where as high heritability coupled with moderate GAM was noticed for traits like plant height (79.00 & 18.16), 100 seed weight (77.42 & 13.87) and plant dry weight (66.31 & 19.64) (Fig 2).

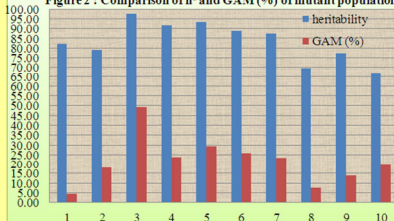
Table 1. Estimation of mean, shift in mean and per cent increase of mean of mutant over control.

Sl. No	Characters	Mean	Shift in mean	% Mean of control
1	Days to flowering	control 54.05 mutant 53.03	-1.02	-1.89
2	Plant height (cm)	control 22.21 mutant 18.24	-3.97	-17.89
3	Number of branches	control 32.70 mutant 37.31	4.61	14.09
4	Plant spread (cm)	control 24.49 mutant 26.27	1.78	7.28
5	Number of pods	control 33.85 mutant 36.86	3.01	8.88
6	Pod yield (gm)	control 23.84 mutant 26.14	2.30	9.64
7	Seed yield (gm)	control 15.24 mutant 17.06	1.83	11.99
8	Shelling percentage (%)	control 63.93 mutant 65.67	1.73	2.71
9	100 seed weight (gm)	control 44.82 mutant 46.26	1.44	3.21
10	Plant dry weight (gm)	control 14.47 mutant 17.12	2.66	18.38

Table 2. Estimates of components of mean, range, coefficients of variability, heritability and GAM for QTs in Bambara groundnut.

Sl. No	Characters	Mean	Max	Min	PCV	GCV	h ²	GAM (%)
1	Days to flowering	53.03	55.20	50.00	2.67	2.42	82.03	4.52
2	Plant height (cm)	18.24	23.25	12.85	11.16	9.92	79.00	18.16
3	Number of branches	37.31	61.40	25.10	24.53	24.22	97.52	49.28
4	Plant spread (cm)	26.27	35.51	15.95	12.65	12.10	91.61	23.86
5	Number of pods	36.86	52.30	18.90	15.28	14.75	93.25	29.35
6	Pod yield (gm)	26.14	37.51	11.96	14.09	13.27	88.74	25.75
7	Seed yield (gm)	17.06	24.26	9.12	12.96	12.11	87.28	23.31
8	Shelling percentage (%)	65.67	76.87	56.64	5.32	4.44	69.59	7.62
9	100 seed weight (gm)	46.26	56.55	38.23	8.70	7.65	77.42	13.87
10	Plant dry weight (gm)	17.12	21.93	8.34	14.38	11.71	66.31	19.64

Figure 2 : Comparison of h² and GAM (%) of mutant population



Discussion

- Moderate PCV coupled with moderate GCV along with narrow difference between PCV and GCV has been observed for all the traits. Therefore this study indicates the extent of greater amount of genetic variability which can be exploited for breeding programme
- Higher heritability coupled with higher GAM was observed for branches per plant, plant height, pods per plant, pod yield per plant and seed yield per plant which indicates the involvement of additive gene effect on expression of traits and effectiveness of selection for these traits (Fig. 2).
- Higher heritability along with low GAM was observed for days to flowering and shelling percentage indicates that their expression was due to the non additive gene action and selection for these traits may not be useful (Fig. 2).

Summary

- M₃ population of SB-42, a variety of Bambara groundnut treated with Ethyl Methane Sulphonate (EMS) studied for genetic variability traits.
- The mean value of mutants were higher than the parent for all the traits except days to flowering and plant height.
- Traits like branches per plant was found to be most affected by mutation.
- Along with branches per plant, the traits like plant height, pods per plant, pod yield per plant and seed yield per plant showed higher heritability coupled with high genetic advance suggests that these traits are governed by additive gene action, therefore such of these traits could be considered for selection

Advisory committee:

Chairperson:

Dr. R Nandini : Assistant professor
Dept. Of GPB, GKVK, UAS, Bengaluru

Members:

- Dr. S Rangaiah
- Dr. A K Chakravarthy
- Dr. N Nagaraju

* Part of MSc (Agri) thesis, to be submitted to University of Agricultural Sciences, Bangalore.

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I INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an important food legume crop grown by resource poor farmers mainly in semi-arid parts of African countries. In much of Africa, Bambara groundnut is the third most important legume after groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Howell 1994). Bambara Groundnut is a member of family Fabaceae and subfamily Faboideae with a chromosome number $2n=22$. It is an autogamous diploid grain legume ($2n=22$) (Howell 1994). It is cultivated principally by farmers as a “famine culture” crop because it has several natural agronomic advantages including high nutritional value, drought tolerance and the ability to produce in soils considered insufficiently fertile for cultivation of other more favoured species such as common beans and groundnuts (Azam-Ali *et al.*, 2001). It is native grain legume of the South Sahara. Botanical studies undertaken by Marechal *et al.*, in 1978 showed great similarities between Bambara groundnut and plant species of the genus *Vigna*. This confirmed the current name *Vigna subterranea* (L.) Verdc proposed by Verdcourt (1980). The common name actually appears to be derived from a tribe “Bambara” who now live mainly in Mali (Goli *et al.*, 1997). According to Swanevelder (1998) there are two botanical varieties reported namely *Vigna subterranea* var *spontanea* which includes the wild varieties and *Vigna Subterranea* var *subterranea* which includes all the cultivated varieties. Bambara groundnut is commonly called by many vernacular names such as Bambara bean, congo goober, ground bean, hog-peanut, Congo groundnut, earth pea, njugo bean, Bambara groundnut, Congo earth pea, kaffir pea, Madagascar groundnut, stone groundnut.

Bambara groundnut is an herbaceous, intermediate, annual legume, and self pollinated with a compact well developed tap root with many short lateral stems on which leaves are borne. Leaves are trifoliate and flowers borne in a raceme (Basu *et al.*, 2007 and Massawe *et al.*, 2002). Its podding habit is either in the soil or on the surface of the ground (Amadou *et al.*, 2001). It can be cultivated up to 1600 m above sea level with an average day temperature of 20–28°C ideal for the crop, it has a growth period of 110–150 days for the crop to develop, Bambara groundnut will grow on any well drained soil but light sandy or loams with a pH of 5.0–6.5 are most suitable (Basu *et al.*, 2007). It fixes atmospheric nitrogen in symbiosis with *Bradyrhizobium* strains through a nodulation process (Gueye *et al.*, 1998). Amadou *et al.*, (2001) stated that Bambara groundnut will often yield well in environments that may be too hostile for more favoured legumes and not prone to the risk of total crop failure, especially in low and uncertain rainfall, making it play an important socio-economic role in the semiarid regions of Africa (Massawe *et al.*, 2002). In this era of global warming and food security threats in world, it will be a crop of hope to alleviate malnutrition and poverty as a result of its drought tolerant characteristics.

The seed is regarded as a completely balanced food because it is rich in iron and protein compared to most of the food legumes. Protein is rich in essential amino acid content like lysine and methionine (Rowland, 1993) and the gross energy value of Bambara groundnut seed is greater than that of several other pulses (Amarteifio *et al.*,

2002). Bambara groundnut grains can be eaten fresh or grilled while immature. Thus Bambara groundnut has a great potential for incorporation into various human foods in India, where it could provide useful plant proteins. Though, it is an important legume crop of semi-arid region, its large scale cultivation is restricted to African countries only. However, attempts are being made to popularise the same in many other parts of the world wherever the climate suits for cultivation of the Bambara groundnut. In India, ten landraces of Bambara groundnut namely, S16-5A, S19-3, SB-4-2, Gabc, DODR-TZ, DODC-TZ, DIPC, Uniswa Red, AS-17, and AHM-753 were introduced in 2002 from the University of Nottingham, UK to the National Research centre for groundnut, Junagadh, Gujarat, India through the National Bureau of Plant Genetic Resources, New Delhi. After being imported, genotypes were tested through grow-out test to avoid the entry of new disease at the Regional Centre of NBPGR, Hyderabad and NRCG, Junagadh. Confirming them free from diseases and pests these were grown in pots as well as in field condition at Junagadh, Gujarat and Hyderabad, Andhra Pradesh where these performed well. Later on these Bambara genotypes were grown at UAS, Bangalore and Bhabha Atomic Research Centre at Mumbai (Singh and Basu, 2004).

In India, the potential areas for the cultivation of Bambara groundnut are Rajasthan, Haryana, Gujarat, Maharashtra, Karnataka and Jharkhand where it can be grown as rain fed crop on the poor fertility soils during rainy season. It can be grown either as sole crop or as intercrop with other legumes and oilseeds. There is thus an urgent need to increase the yield through developing high yielding varieties, breeding for disease and pest resistance, proper package of practice, improvement of the nitrogen-fixing capacity and a need for development of stable varieties for new areas (Singh and Basu, 2004). The yield potentials of Bambara groundnut is restricted, because of autogamous nature of the crop, hybridisation is difficult with several attempts of artificial hybridisation reported as unsuccessful (Suwanprasert *et al.*, 2006) limiting genetic variability for quantitative characters. Hence the genetic improvement of the crop was attempted through mutation breeding. Mutation breeding has been considered as one of the promising short-cut method of breeding which could be useful for the development of the desired varieties of different crop plants. Induced mutations are necessary to enhance rate of genetic variability since spontaneous mutation rate is very slow and that prevents breeders to exploit them in plant breeding programs. Bambara groundnut natural germplasm do not carry any gene for earliness, double seeded pods and mosaic virus disease resistance. In order to fulfil our desired plant ideotype, mutation breeding is considered to play important role in creating genetic diversity within short period of time. Adu-dapaah *et al.* (2003) have reported improvement of Bambara groundnut productivity through induced mutation using EMS and gamma radiation and developed mutants resistance to *Cercospora*, *Anthraco* and viruses. Correlation co-efficient reveals the existence of association between the characters. As the indirect association of characters increases it is difficult to explain a system of correlation. The method of path-coefficients developed by Wright (1921) is helpful in assessing whether association of characters with yield is due to their direct effect on yield, or it is a consequence of their indirect effect through some other traits. However, the inheritance of quantitative characters is often influenced by variation in other characters which may be due to pleiotropy or genetic linkage (Horland, 1939). Therefore, the knowledge of character association helps in

determining the extent of improvement that could be brought about in the component characters and in selecting suitable and high yielding genotypes.

Thottappilly and Rossel (1997) reported eight viruses infecting Bambara groundnut in farmer's fields in Nigeria: cowpea aphid-borne mosaic potyvirus, blackeye cowpea mosaic potyvirus, peanut mottle potyvirus, cowpea mottle carmovirus, cowpea yellow mosaic virus, cowpea mild mottle carlavirus, cucumber mosaic cucumovirus and southern bean mosaic sobemovirus. The incidence of yellow mosaic virus disease is major constraint in cultivation of *Vigna spp* in all the parts of India; it causes significant loss to the Indian economy every year. Therefore, there is a necessity for development of yellow mosaic virus resistant genotypes. In Bambara groundnut also yellow mosaic virus disease was reported by Muniyappa and Veeresh in (1984) from the field of Agriculture farm, University of Agriculture Sciences, Bangalore and confirmed its transmittance principally by whitefly, *Bemisia tabaci* with characteristics of faint yellow discolouration of younger and bright yellow colouration older leaves. Smita (2012) also reported similar findings. Her studies indicated susceptible and resistant mutant lines for the yellow mosaic virus disease transmitted through whitefly *Bemisia tabaci* in the gamma irradiated mutant population under natural condition. In recent years, DNA markers are extensively used for tagging of disease resistant genes in marker-assisted plant breeding. Indirect selection using DNA markers linked to resistance genes could be one of the alternate approaches as they enable marker assisted selection (MAS) to overcome the inaccuracies in the field evaluation (Selvi *et al.*, 2006). The use of molecular markers for resistance genes is particularly powerful as it removes the delay in breeding programmes associated with the phenotypic analysis (Karthikeyan *et al.*, 2012). This is possible due to availability of many kinds of markers *viz.* RAPD, AFLP and SSR that are used for genotyping purposes. Realising the importance and need for such a comprehensive study in Bambara groundnut, the present investigation was undertaken with the following objectives.

1. Studies on variability parameters of different biometric traits in M₃ generation.
2. To estimate the correlation and path coefficient between seed yield and yield component characters.
3. Molecular screening of Bambara groundnut mutants for yellow mosaic virus resistance using heterologous probes.

II REVIEW OF LITERATURE

A thorough understanding of the genetic variation for different traits, character association, direct and indirect effects of components of grain yield and yellow mosaic virus disease resistance in the population are important for any crop improvement programme to be successful. The available literature on Bambara groundnut and other related species have been reviewed under the following headings.

2.1 Genetic variability studies for different biometrical traits in mutant population

2.2 Correlation studies for yield and its attributing traits

2.3 Path Coefficient analysis for yield and its attributing traits

2.4 Molecular screening for yellow mosaic virus disease resistance

2.1 Genetic variability studies in mutant population of Bambara groundnut (*Vigna subterranea* (L.) Verdc.)

Increasing yield is always the paramount aim of any breeder. However, assessing factors responsible for increasing yield is always difficult. Yield is the end product of action and interaction of vital activities of plant throughout the life cycle and is controlled by numerous factors shaped by genetics and environment. Among these factors, most important is the inherent potential of the plant to produce higher yield which depends upon the hereditary make up of the plant. Therefore, for rational improvement of crop, understanding of the magnitude of genetic variability and the extent to which the desirable characters are heritable becomes essential. The determination of genetic variability and its partitioning into various components, in any crop, is necessary to have an insight into the genetic nature of yield and its components. Thus, the magnitude of heritable variation, particularly its genetic components, is clearly most important part of the breeding material which has close bearing on its response to selection.

Adu-Dapaah *et al.* (2003) initiated the improvement of Bambara groundnut productivity using mutation breeding and considered two varieties Tom and Nav treated with gamma rays ranging from 50 to 400 Gy and with EMS at concentrations from 0.2 to 1.2 %. Genetic variation was reported up to four times, than the control, was estimated for both gamma irradiation and EMS treatment compared to the untreated controls. Selected mutants outperformed the untreated control plants in most of the agronomic traits.

Adu-Dapaah and Sangwan (2004) reported that gamma irradiation induced higher genetic variation of up to four times within the varieties used in the study compared to the unirradiated control and they also suggested that Bambara groundnut yield could be

increased through selection for number of pods per plant using the *in vitro* plus *in vivo* system and embryo axis explants. Over four generations per year were obtained compared to one or two generation in field condition. All the plants were morphologically normal and fertile. The shorter duration, high efficiency and genotype independency makes this system well suited for wider biotechnological applications in Bambara groundnut.

Spreeth and De Ronde (2004) evaluated M₂ to M₅ generation plants. The M₂ to M₄ plants were evaluated both in the greenhouse and the field condition. Three Bambara groundnut mutants yielded more than the parent, and one showed relatively high yield under drought stress. It proved possible to examine mutant plants at the seedling stage in wooden boxes, mature plants were screened in rain-out shelter and physiological traits were distinguished among the tested lines for drought stress.

Vijaykumar (2011) induced variability using gamma irradiation in variety SB-42 of Bambara groundnut with doses of 150Gy, 175Gy, 200Gy and 225Gy and observed that in M₁ generation lower dose of 150Gy did not affect the germination whereas germination was affected at 225Gy. Optimum dose was found to be 200Gy for survival. In M₂ generation, negative shift in mean was observed for all the characters except days to flowering, but shelling percentage and number of pods per plant showed positive shift in mean at 150Gy and 175Gy.

Smita (2012) conducted variability studies in M₃ generation and revealed that negative shift in mean for all the characters except, for shelling percentage and 100 seed weight and increased variability for all the characters like plant height, branches per plant, plant spread, pod yield, seed yield, number of pods per plant, 100 seed weight and shelling percentage as compared to control. She also reported that high heritability and high genetic advance as *per cent* mean were observed for all the characters except for plant height.

2.2 Induced mutation studies in other pulse crops

2.2.1 Mung bean (*Vigna radiata* (L.) wilzek)

Mohd Rafiq and Samiullah (2006) observed a significant increase in mean values of the fertile branches per plant, pods per plant and seed yield per plant in mutants of mung bean. They reported that genetic parameters for the yield and its components were higher than the control in two generations and increased genetic variability for these traits provides a greater scope for further selection.

Tah (2006) reported that environmental stress due to application of epigenetic mutations have proved to be less stable than normal DNA sequence alterations in the study of mung bean and observed the wide range of genetic variation in the morphological parameters was associated with the higher doses of mutagens.

Singh and Rao (2007) irradiated two green gram cultivars (TARM-1 and Sujata) by gamma radiation (30, 40, 50 and 60 Kr) and studied different morphological mutations. They reported large spectrum of morphological mutations in these cultivars and the frequency of morphological mutation showed positive relation with the dose of radiation. Effect of mutagen was found to be more in Sujata than the variety TARM-1.

Imran *et al.* (2011) reported the effect of EMS on the two species of *Vigna* i.e *Vigna mungo* and *Vigna radiata* in M₁ and M₂ generation for isolation of novel mutants and their respective comparative studies. A better variant dose of EMS was found to be very effective in generating high yielding mutants in both the species.

Rajibet *al.*(2012) inferred pure line seeds of *Vigna radiata* were treated with ethyl methane sulphonate (EMS) and analysed the genetic variability and heritability traits in M₂ generation. The analysis variance revealed highly significant differences among the studied characters and the increased genetic variability in treated population has a high scope for selection and can be exploited for the further improvement of mung bean.

Imran *et al.* (2014) studied genetic diversity in M₃ generation of *Vigna radiata* in response to gamma radiation. They observed higher heritability and significance correlation, and indicated that germination percentage, 100-seed weight and seeds per pod may be used to select higher yielding mung bean genotypes.

2.2.2 Black gram (*Vigna mungo*(L.). hepper)

Deepalakshmi and Kumar (2004) reported viable macro mutants with changes in traits, like height, maturity, leaf, pod, seed and sterile mutants in the M₂ generation. They found that, the mutation spectrum was much wider at 20 Kr and narrow at 70 Kr. In common, the mutation frequency was high on M₁ plant than M₂ plant in mutagens.

Sharma *et al.*, (2005) treated the seeds of black gram (*Vigna mungo*) cultivars PDU-1 and T-9 with gamma rays at different doses to determine their mutagen sensitivity, LD₅₀, mutagenic effectiveness and efficiency. Dose of gamma rays decreases the germination, plant survival and pollen fertility. The LD₅₀ values revealed that PDU-1 was more radio-resistant than T-9. The mutation frequency of gamma rays was less in PDU-1 than in T-9. The lower doses of mutagens were more effective and efficient than the higher doses.

Arulbalachandran and Mullainathan (2009) studied genetic variation among four mutants with parent of black gram. Increased variability was observed for pod yield per plant, seed yield and number of pods per plant. Some mutants showed superior in traits like high protein content, lodging resistance, and high seed yield compared with parent cultivars.

Sri devi and Mullainathan (2012) conducted a study on effect of gamma rays and ethyl methane sulphonate (EMS) on mutagenesis of black gram to determine the effects

of induced mutation on the species. Mean performance of different quantitative traits was significantly better when compared with the control. High values of heritability and genetic advance indicate the possibility of inducing desirable mutations for polygenic traits accompanied by effective selection in M_3 and later generations.

2.2.3 Cow pea (*Vigna unguiculata* (L.) Walp)

Henry (2002) induced variability using gamma radiation in Charodi-1 a cultivar of cowpea, having small to medium seeds with brown seed coat. The mean performance of white seed-coat mutants CAZC-3, CAZC-W, CAZC-9 and CAZC-11 had high yield potential with higher number of pods per plant than the parent cultivar and standard control cultivar and also revealed that mutant CAZC-10 flowered and matured earlier than mutant CAZC-10 flowered and matured earlier than the control cultivars GC-3, V-240 and V-585.

Pandey and Dhansekhar (2003) irradiated cowpea accessions with gamma rays. The mutant line with reduced plant height showed superiority in pod number, 100 seed weight and seed yield per plant. Both mutant and parents matured in 62 days and when compared to V-130, the mutant was found far superior in respect of earliness, seed size yield and reduced plant height.

Adekola and Oluleye (2007) irradiated dry seeds of cowpea variety IT-84S2246D to gamma irradiation. Mutants were evaluated for useful agronomic traits in M_2 and the selected mutants were found to possess useful agronomic traits like increased yield, easy harvesting and insect tolerance hence experiment suggested that there is possibility for further cowpea improvement through induced mutation.

Manggoel *et al.* (2012) estimated genetic variability, heritability of some reproductive characters of 10 cowpea accessions and reported significant variability for days to 50% flowering, number of peduncles per plant, flowers per plant, pods per plant, seeds per pod per, pod length, 100 seed weight and grain yield. Phenotypic coefficient of variation and genotypic coefficient of variation were high for the traits studied except pod length and seeds per pod. High broad-sense heritability indicated the presence of additive gene effects.

Hornand Shimelis (2013) conducted experiment to determine an ideal dose of gamma radiation to induce genetic variation in selected cowpea (*Vigna unguiculata*) genotypes. Their results revealed that, using linear regression model the LD50 for genotypes Nakare, Shindimba and Bira calculated was 165.24, 198.69 and 689 Gy respectively and These findings may assist as reference doses for large-scale gamma irradiation of cowpea genotypes to induce genetic variation.

Singh *et al.* (2013) conducted genetic variability studies in cowpea variety RC 19 treated with gamma rays and ethyl methane sulphonate (EMS) in M_2 generation. Their results indicated significant differences in the mean value of many characters like plant

height, number of primary branches, number of pods, number of grains, 100-seed weight and reduced seed yield significantly with increasing concentration of both the mutagens. The study revealed that magnitude of phenotypic coefficient of variation was distinctly high for plant height, number of pods per plant, seed yield per plant, biological yield, harvest index and protein content.

2.2.4 Lentil (*Lens culinaris* Medik.)

Rajput *et al.*, (2001) conducted induced variability study on M₂ generation of two varieties of lentil and selections were made on the basis of earliness, plant height, branches/plant and 100 grain weight. The lines AEL 49/20/91 and AEL 13/30/91 showed positive result in 100 grain weight and some agronomic characters were superior over the parent varieties. They concluded as the mutant lines AEL 13/30/91 and AEL 49/20/91 to serve as potential lentil genotypes in future.

Shahnawaz and Samiullah khan (2014) studied induced variability of yield characteristics in lentil (*Lens culinaris* Medik.) var. DPL-15 by using chemical mutagen methyl methane sulphonate (MMS). Quantitative characters were studied in M₂ generation like branches per plant, fertile branches per plant, pods per plant and 100 seeds weight. The shift in mean values in positive direction for 100 seeds weight indicates that selection for this character would be effective in subsequent generations.

2.3 Correlation between yield and yield components

The attainment of characteristic form and function in crop plants depends upon a chain of interrelated events which are sequential in time. Moreover, these events do not occur haphazardly but follow an integrated pattern. Seed yield is an obvious example of such integration and its expression is dependent upon action and integration of various components. As Grafius (1956) has suggested, there may not be genes for yield *per se* but, there are genes for various yield components.

Association between quantitative characters statistically determined by Pearson's correlation coefficient (r) has been quite useful as basis for selection. The basic concept of correlation was elaborated and discussed by Fisher (1918 and 1936) and Wright (1921) for plant breeding programmes.

2.3.1 Correlation studies in Bambara groundnut (*Vigna subterranea* L. Verdc)

Karikari (1972) found that seed yield was significant and positively associated with the days to germination, maturity, number of pods per plant, number of seeds per pod and 100 seeds weight. Number of stems per plant and 100 seed weight were found to be used for further selection.

Ofori (1996) observed large proportion of variation in number of pods per plant, 100-seed weight, number of seeds per plant, leaf area, number of leaves per plant and

canopy spread were attributable to genotypic differences in the population. The genotypic correlations were higher than the phenotypic correlations. Direct and indirect effects of seed yield components on seed yield were similar for both bunch and spreading types. The relationship between pod yield and leaf number appeared to depend on the degree of determinacy.

Goli *et al.* (1997) reported characters like number of leaves, number of pods, shell thickness and 100 seed weight were strongly correlated with grain yield.

Makanda *et al.* (2009) performed correlation analysis on yield traits in Bambara groundnuts varieties. They considered characters like Days to 50% emergence, days to 50% flowering, days to 95% physiological maturity, number of plants at harvest, number of pods per plant and dry pod yield per plot were measured and analysed using GenStat. Most of the correlation coefficients noticed were significant ($P < 0.05$).

Jonah *et al.* (2010) noticed the correlations between the seed yield and other quantitative traits. Positive correlation was reported between seed yield per hectare and pod yield per plant. There was a significant genotypic and phenotypic correlation coefficient in association between pod length and pod width, seed length and seed width, which could serve as a good selection indices for high yielding cultivars.

Benedict and Micheal (2011) studied interrelations between pod and seed yield traits in Bambara groundnut. The correlation coefficient of seed yield per plant with number of flowers per plant and number of pods per plant sign showed significantly positive in both early and late planting dates.

Vijaykumar (2011) studied gamma irradiated variability studies in Bambara groundnut. In M_2 generation, he reported that seed yield was positively correlated with the traits like pod yield, shelling percentage and number of pods per plant at all the doses and concluded that by selection of these characters improve yield.

Jonah (2012) studied the twelve cultivars of Bambara groundnut and observed that, a positive correlation and statistically significant correlation coefficients between pod yield/plant and seed yield/h revealing that the more the pod numbers the higher the yield of Bambara groundnut per hectare. This provides that pod yield/plant could respond to selection in Bambara groundnut. Similarly, the correlation between pod width and pod length seed width and seed length were positive and significant. Furthermore, pod length had a positive and statistically significant correlation coefficient with 100 seed weight, pod width, seed width and seed length.

Smita *et al.* (2014) reported that the seed yield was strongly correlated with the plant height, branches per plant, plant spread, pod per plant, pod yield and plant dry weight and these traits could be selected for improvement in yield in M_3 generation of SB-42 Bambara groundnut genotype.

Maunde *et al.* (2015) found the seed yield had strong positive correlation with the number of pods per plant, 100 grain weight and harvest index in the “mubi” cultivar of Bambara groundnut.

2.3.2 Correlation studies in related pulses

2.3.2.1 Mung bean (*Vigna radiata* (L.) wilzek)

Singh *et al.* (2009) studied the correlation on eighty genotypes of mung bean and noticed that the seed yield per plant exhibited highly significant and positive phenotypic correlation with biological yield per plant and harvest index. The phenotypic correlations of seed yield with remaining characters were found to be either negative or positive but non-significant.

Aijaz Ahmad *et al.* (2013) studied the inter-relationship among yield and its contributions in 35 mung bean genotypes. Their results indicated that seed yield per plant showed positive significant association with number of clusters per plant, number of primary branches per plant, number of pods per plant, number of seeds per pod, pod length, and 100-Seed weight, and negative and significant association with days to 80 % maturity.

Narasimhulu *et al.* (2013) reported that seed yield had positive and significant correlation with number of pods per plant, clusters per plant, number of pods per cluster and biological yield per plant in forty genotypes of mung bean.

Correlation coefficient analysis on 54 genotypes of mung bean for eight characters was conducted by Muzibul Aloma *et al.* (2014) and reported that 100 seed weight showed significant positive relationship with seed yield.

2.3.2.2 Black gram (*Vigna mungo* (L.) hepper)

Govindaraj and Subramanian (2001) reported that primary branch number, cluster number, pod number, pod length and seed number per pod had strong positive association with yield in black gram, besides, their inter correlation were positive and significant.

Nagarjuna *et al.* (2001) inferred character association analysis in fifty genetically diverse genotypes of black gram revealed positive significant association of biological yield per plant, pods per plant, clusters per plant, branches per plant, plant height, harvest index and days to maturity with grain yield in decreasing order of their magnitude.

Parameswarappa and Kumar (2005) studied character association in 40 black gram genotypes and results are showed that pods per plant, number of branches per plant and plant height were significantly and positively associated with yield per plant.

Chauhan *et al.* (2007) assessed two hundred ten divergent genotypes of urd bean for association between seed yield and component characters. A significant and strong association existed between seed yield, pods per plant, number of clusters per plant, biological yield per plant, number of seeds per pod, harvest index, plant height, number of pods per cluster and number of primary branches per plant.

Shivade *et al.* (2011) observed that the plant height, number of branches per plant, number of clusters per plant, number of pods per plant, number of pods per cluster, length of pod, number of seeds per pod and dry matter per plant had strong positive association with seed yield per plant at both the phenotypic and genotypic level in thirty six black gram genotypes.

Ishaparveen *et al.* (2011) estimated the genotypic and phenotypic correlations in eight parents and their 28 F1 crosses of black gram. Their results showed that significant positive association of pods per plant, harvest index and clusters per plant with seed yield in parental generation where as in F1 generation, significant positive association of clusters per plant, pods per plant, days to maturity, days to 50% flowering, pods per cluster and 100-seed weight with seed yield.

Punia *et al.* (2014) studied the correlation on 133 accessions of urd bean for eight quantitative characters and noticed that seed yield per plant showed strong positive correlation with number of pods per plant and number of branches per plant and strong negative correlation with days to fifty per cent flowering and days to maturity.

2.3.2.3 Cowpea (*Vigna unguiculata* L. Walp)

Manggoel *et al.* (2012) conducted experiments to estimate genetic variability, heritability, correlations and path analysis of some reproductive characters on grain yield of 10 cowpea accessions. Positive correlation was found between grain yield and pods per plant, number of peduncles per plant, flowers per plant and 100-seed weight and these traits were considered as selection criteria for obtaining good parental lines in cowpea breeding programs.

Om Vir and Singh (2014) concluded that number of seeds per pod, number of pods per plant, number of pods per cluster, number of cluster per plant, days to 50% flowering and days to maturity had positive and significant correlations with seed yield per plant in 33 indigenious and exotic accession of cowpea.

Diriba *et al.* (2014) reported positive and significant environmental correlation between seed yield and number of primary branches per plant, number of secondary branches per plant, days to 50% flowering, number of pods per plant, number of seeds per pod and plant height in cowpea.

2.4 Path co-efficient analysis for yield and its attributing traits

Path coefficient analysis provides an effective means of partitioning direct and indirect causes of association with yield. It also reveals the magnitude of contribution made by different plant characters towards yield thereby imparting confidence in selection of important yield attributes. The method of path coefficients proved useful in analyzing correlation coefficient in this system of related variables. Path coefficient analysis was insinuated by Wright (1921) and its practical utility was demonstrated by Dewey and Lu (1959). The review of the work done utilizing path coefficient analysis in Bambara groundnut and related species are presented below.

2.4.1 Path coefficient analysis in Bambara groundnut (*Vigna subterranea* L. Verdc)

Ofori (1996) found the indirect and direct effects of seed yield components. The 100-seed weight and number of pods per plant had direct positive effects on seed yield and the number of pods per plant gave the best indication of seed yield status. In bunch type number of leaves was more important in determining seed yield, but leaf size in the spreading types. Selection for plants with high and stable number of pods per plant must improve production level of Bambara groundnut.

Misangu *et al.* (2007) reported that Path coefficient analysis shows that leaf length, number of days to flowering, Petiole length on yield was far less than its respective residual effects. Their investigation showed that number of pods interacted negatively with 100 seed weight in influencing yield of Bambara groundnut. Taller plants, longer leaves and more podding are variables which are related to higher yields of Bambara groundnut under green house condition.

Adeniji *et al.* (2008) reported in Bambara ground that, number of leaves per plant had direct effect on pod yield, reveals its influence on pod yield, while the number of root nodules showed the indirect effect on pod yield and its largest indirect effect from plant dry weight. The path analysis revealed the adverse effect of number of pods per plant on pod yield/ha was largely due to indirect effect of plant height at flowering, plant dry weight and number of effective root nodules. The seed weight had the largest influence on seed yield/ha.

Makanda *et al.* (2009) performed path coefficient analyses in Bambara groundnuts cultivars on yield component traits. Dry pod yield was largely dependent on the direct effects of its component traits compared to the indirect effects through other traits, reveals that direct selection is more effective. All the direct effects showed contrasting influences on dry pod yield for the same traits, but at different planting dates except for the number of plants per plot on which they were all positive. Indirect effects through number of plants per plot were high, suggesting the possibility of correlated response in selection.

Jonah *et al.* (2010) found the correlations between seed yield and other quantitative traits. The path coefficient analysis of traits revealed that the seed yield indicated positive direct effect on traits like pod length, plant emergence during vegetative phase. Even though traits noticed a positive but a non-significant genotypic correlation coefficient of seed yield with other traits suggesting the inefficiency of selection based on correlations alone.

Vijaykumar (2011) reported that traits showing high positive correlation with yield may not show direct positive effect on the yield trait. Shelling percentage, Pod yield per plant and number of pods per plant showed large direct effect on pod yield in all the treatments. Those traits which showed direct positive effect on the yield like shelling percentage, pod yield per plant and numbers of pods per plant could be selected for improvement in yield of Bambara groundnut.

Smita *et al.* (2014) performed path analysis, indicated that except for plant height and number of branches per plant all other characters had positive direct effect on seed yield. Pod yield had highest indirect effect on seed yield. Those characters which showed significant positive correlation and exerted direct positive effect on the yield like pod yield per plant, shelling percentage and numbers of pods per plant could be selected for improvement in yield in M₃ generation of SB-42 Bambara groundnut genotype.

Maunde *et al.* (2015) found the seed yield had strong positive correlation with the number of pods per plant, 100 grain weight and harvest index in the “mubi” cultivar of Bambara groundnut. Although only number of pods per plant and harvest index showed direct effect on seed yield, 100 seed weight noticed very less effect on the seed yield.

2.4.2 Path coefficient analysis in related pulses

2.4.2.1 Green gram (*Vigna radiata* L. wilczek)

Mallikarjuna Rao *et al.* (2006) studied path coefficient analysis in 60 mung bean genotypes and results are indicated that pods per plant, biological yield and harvest index had maximum direct contribution on seed yield.

A path analysis study made by Singh *et al.* (2009) with eight mung bean germplasm in three environments revealed that cluster per plant followed by biological yield per plant, 100 seed weight, days 50% per cent flowering and seeds per pod exerted high positive direct effects on seed yield, therefore they concluded that these are the most important criteria for seed yield improvement in three environment and high order negative direct effects on seed yield per plant were exhibited by pods per plant, plant height.

Aqusa *et al.* (2010) carried out the path coefficient analysis in ten mung bean genotypes to establish the degree to which various plant traits associate with seed yield. Positive direct effects were exerted through secondary branches, pod length, pods per

plant, 100 seed weight, harvest index and total plant weight while primary branches, clusters per plant, plant height and pods per cluster had negative direct effects.

Aijaz Ahmad *et al.* (2013) conducted experiment on path coefficient analysis in 35 mung bean genotypes and their results revealed that maximum positive direct contribution to seed yield per plant came from number of seeds per pod (0.424) followed by 100-Seed weight (0.419, number of pods per plant (0.339) and number of primary branches per plant (0.177).

Muzibul Aloma *et al.* (2014) conducted path coefficient analysis on 54 genotypes of mung bean for eight characters concluded that pods per plant contributed maximum direct effects having positive on seed yield. Plant height, pod length and 100 seed weight also had positive direct effect on seed yield.

2.4.2.2 Black gram (*Vigna mungo* L. Hepper)

Govindaraj and Subramanain (2001) performed path analysis in black gram which indicated that the contribution of pod number followed by seed number per pod and number of primary branches was much through their direct effects and their indirect effects by way of other traits were also much pronouncing.

Nagarjuna and Reddi Sekhar (2001) reported that biological yield per plant harvest index and pods per plant had high direct effects on grain yield, where as high indirect positive effects were shown by other traits through biological yield per plant, harvest index and pods per plant in black gram.

Parameswarappa and Kumar (2005) showed that Pods per plant had the highest positive direct effect on yield followed by test seed weight and number of branches. They concluded that simultaneous selection of plant height, number of branches per plant and number of pods per plant together will also be useful in bringing higher yields in black gram.

Chauhan *et al.* (2007) concluded path analysis in 110 divergent genotypes of urd bean. Their study revealed that very high positive direct contribution of number of pods per plant, number of seeds per plant, 100 seed weight and biological yield per plant towards the expression of seed yield per plant.

Shivade *et al.* (2011) conducted path coefficient analysis with sixty genotypes of black gram and reported that selection based on number of cluster per plant, number of pods per plant, dry matter per plant, plant height, number of seeds per pod, length of pod and number of pods per cluster could help in genetic improvement of seed yield per plant in black gram.

Ishaparveen *et al.* (2011) studied path analysis in eight parents and their 28 F1 crosses for twelve characters and results are revealed that number of seeds per cluster,

harvest index, pods per plant, and number of primary branches per plant showed high positive direct effects on seed yield in parents where as in crosses, seeds per pod, pods per plant harvest index and days to maturity showed high positive direct effect on seed yield. Hence selection based on these traits would be effective in increasing the seed yield.

Pushpareni *et al.* (2013) studied path coefficient analysis that, revealed that the highest positive direct effect on grain yield per plant was recorded by pods per plant followed by 100 seed weight, days to maturity and days to 50% flowering. Hence these traits could be used for the improvement of grain yield in the evolution of high yielding varieties of black gram.

Punia *et al.* (2014) studied path coefficient analysis on 133 accessions of urd bean for eight quantitative traits and results are revealed that number of pods per plant and number of branches per plant had major direct and indirect effect on yield contributing traits in urd bean.

2.4.2.3 Cowpea (*Vigna unguiculata* L. Walp)

Diriba *et al.* (2014) performed path analysis and revealed that, yield per plant exerted the maximum positive direct effect on seed yield followed by number of pods per plant, while number of secondary branches per plant, days to flowering, days to maturity and number of seed per pod exhibited negative direct effect phenotypically. In addition, genotypic path analysis revealed that, maximum direct effect on seed yield was exerted by number of pods per plant and yield per plant. However, days to 50% flowering, days to maturity, number of secondary branches per plant and number of seed per pod exerted negative direct effect on seed yield. Thus, yield per plant and number of pods per plant could be used as a selection index for cowpea improvement.

2.5 Molecular screening for yellow mosaic virus disease resistance in pulse crops

Genetic variability or diversity in plants has been in traditionally assessed by morphological or bio-chemical traits. Assessment based on phenotype may not be reliable because of the influence of environment on gene expression. The molecular markers allow direct assessment of variation in genotypes not only within a species but also across species. However, the type of molecular method used to measure genetic difference being assessed.

Muniyappa and Veeresh (1984) reported that yellow mosaic virus disease caused by the horse gram yellow mosaic virus in Bambara groundnut and transmitted by whitefly (*Bemisia tabaci*) during the summer of 1981 at agriculture farm, UAS, Bengaluru.

Thottappilly and Rossel (1997) reported eight viruses infecting Bambara groundnut in farmer's fields in Nigeria: cowpea aphid-borne mosaic potyvirus, blackeye

cowpea mosaic potyvirus, peanut mottle potyvirus, cowpea mottle carmovirus, cowpea mosaic comovirus (cowpea yellow mosaic virus), cowpea mild mottle carlavirus, cucumber mosaic cucumovirus and southern bean mosaic sobemovirus.

Basak *et al.* (2004) developed YMV-tolerant lines from a single YMV-tolerant plant identified in the field within a large population of the susceptible cultivar T-9, were crossed with T-9, and F1, F2 and F3 progenies raised. The selected F2 and F3 plants were screened by using 24 RGA primers, only one pair, RGA 1F-CG/RGA 1R, was found to be polymorphic among the parents. Selected F2 individuals and F3 families were genotyped with the polymorphic RGA primer pair and the polymorphism was found to be linked with YMV-reaction. This primer pair amplified a 445bp DNA fragment only from homozygous tolerant and the heterozygous lines. The 445bp marker band was sequenced and named 'VMYR1'. The predicted amino acid sequence showed highly significant homology with the NB-ARC domain present in several gene products involved in plant disease resistance.

Reddy (2009) isolated yellow mosaic virus disease resistance mutant line in *Vigna radiata* by recurrent gamma irradiation with the dose of 600 Gy earlier and 500Gy in M₃ generation and mutant was purified by growing up to M₃M₆ generations. All the mutant plants showed very minor leaf symptoms but no symptoms in the pod but the susceptible plants showed leaf and pod symptoms and showed severe yield losses. This mutant was used in crossing program to study the genetics of YMV resistance.

Soumitra *et al.* (2011) developed molecular markers linked to mung bean yellow mosaic Indian virus (MYMIV) resistance to facilitate genotyping of urd bean and mung bean germplasms for MYMIV-reaction. Resistance-linked molecular markers were successfully developed from consensus motifs of other resistance (R) gene or R gene homologue sequences. Two MYMIV-resistance marker loci, YR4 and CYR1, were identified and of these two CYR1 are completely linked with MYMIV-resistant germplasms and co-segregating with MYMIV-resistant F2, F3 progenies of urd bean. They demonstrated that YR4 and CYR1 markers could be efficiently employed together in a multiplex- PCR-reaction for genotyping both *V. mungo* and *V. radiata* germplasms.

Gioi *et al.* (2012) studied the linkage of SSR markers to cowpea yellow mosaic virus resistance using 20 CYMV resistance lines and 20 susceptible lines of cowpea. Among 60 SSR primers used to screen polymorphism between stable resistance (GC-3) and susceptible (Chrodi) genotypes, only 4 primers were polymorphic. These 4 SSR primer pairs were used to detect CYMV resistant genes among 40 cowpea genotypes. The polymorphism obtained was used to analyse the association of the linkage markers with CYMV resistant trait using QTXb20 software.

Praveen and Savithamma (2013) studied random amplified polymorphic DNA (RAPD) marker associated with mung bean yellow mosaic virus (MYMV) resistance in mungbean (*Vigna radiata* (L). Wilczek) by employing bulk segregant analysis in

recombinant inbred lines (RILs). Out of 20 random decamers ten primers like OPA-03, A-06, A-03, OPA-09, A-09, OPB 7, UBC-391, OPC-08, UBC 499 and A-04 showed polymorphism between parents Chinamung and BL 849. UBC 499 amplified a single 700 bp band in the genotype BL 849 and MYMV resistant bulk which was absent in Chinamung and MYMV susceptible bulk. PCR amplified 700 bp marker band was detected in individuals of F5 RILs constituting the MYMV resistant bulk indicating that primer UBC 499 was linked to MYMV resistance.

Bhupender *et al.* (2014) collected 500 soybean germplasm lines from different parts of the world were screened for YMV disease reaction at YMV hotspot consecutively for 3 years. It could identify 96 genotypes, comprising 48 each of resistant and susceptible genotypes that showed stable disease reactions over the years. Soybean 'R' gene-based primer pair linked with YMV variation in *Vigna mungo* failed to differentiate the YMV resistant and susceptible soybean genotypes. Genetic diversity panel of the 96 soybean genotypes was analyzed with 121 simple sequence repeat (SSR) markers, of which 97 were polymorphic (80.16% polymorphism). Total of 286 normal and 90 rare alleles were detected, with a mean of 2.36 and 0.74 alleles per locus, respectively. The findings of this study strongly suggested the use of exotic collections that were found resistant to YMV for genetic enhancement of soybean.

III MATERIAL AND METHODS

The present investigation was carried out at K-block, University of agricultural Sciences, GKVK, UAS, Bengaluru located at an altitude of 899 m above Mean Sea Level and at 13°00'North latitude and 77°35'East longitude, which falls in Agro-climatic zone 5 of Karnataka. The soil is red sandy loam and is characterized by low water and nutrient holding capacity and low in productivity (<http://www.uasbangalore.edu.in/research/agrometeorology>). In general the soil texture is grouped under silty-clay-loam to clayey with pH around 6.5.

The details of the plant material and methods followed in the experiments conducted are presented and information on protocols and statistical tools used for analysis are also presented in the respective experiments.

- Studies on variability parameters in M_3 generation.
- Screening for yellow mosaic virus disease resistance in M_6 generation using SSR markers.

3.1 Experiment I - Studies on variability parameters in M_3 generation

3.1.1 Experimental materials

Bambara groundnut variety 'SB-42' was used in present investigation. The present experiment-I involved seeds of M_3 generation developed using 0.3 % of Ethyl Methane Sulphonate (EMS) forwarded by the Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru.

3.1.2 Method

Seeds of M_3 generation were sown in plant to progeny row in a single replication along with parent (SB- 42) with spacing of 30 cm between rows and 10 cm within a row at K-block, GKVK, Bengaluru. N: P: K fertilizers dose of 25:75:38 kg per hectare was applied and the field was irrigated once in a week. Suitable crop protection measures were taken during the period of the crop growth.

3.1.3 Following observation were recorded in M_3 generation

3.1.3.1 Plant height (cm)

Length of the main branch of each plant was measured from the base of the plant (ground level) to the upper most tip of the main stem at maturity, expressed in centimetres.

3.1.3.2 Plant spread (cm)

Plant spread of each plant was measured as the average of the two canopy diameter at right angles to each other and expressed in centimetres.

3.1.3.3 Number of branch per plant

The number of branches in each plant was counted and recorded at the maturity.

3.1.3.4 Days to flowering

Number of days taken from date of sowing to the days when plants showed 50 per cent flowering was recorded.

3.1.3.5 Number of pods per plant

The number of pods in each plant was counted at maturity.

3.1.3.6 Pod yield per plant (g)

All the pods of each plant were weighed after optimum drying of the pods was expressed in grams.

3.1.3.7 Seed yield per plant (g)

Weight of total number of seeds per plant was recorded in grams

3.1.3.8 Shelling percentage

Shelling percentage was calculated as the ratio of weight of the dry seeds to the weight of the dry pods and expressed in percentage.

$$\text{Shelling (\%)} = \frac{\text{Weight of the dry seed (g)}}{\text{Weight of the dry Pod (g)}} \times 100$$

3.1.3.9 Hundred seed weight (g)

100-seed weight was estimated by weighing 100 clean and uniform seeds picked randomly from each genotypes and the weight was recorded in grams.

3.1.3.10 Plant dry weight (g)

Biological yield produced by each plant after drying was recorded in grams.

3.1.4. Statistical analysis in M₃ generation;

In M₃ generation all quantitative characters viz., plant height, plant spread, number of branches, days to flowering, number of pods per plant, pod yield, seed yield, shelling percentage, 100 seed weight, and plant dry weight were recorded and genetic variability, correlation and path coefficient for these characters was worked out.

3.1.4.1 Mean

On the basis of individual plant observation, the mean value for each character in all the population was computed as follows

$$X = \Sigma X_i / n$$

Where,

X = Mean

ΣX_i = Sum of all the sample observations

X_i = Individual value

n = Number of sample observation

3.1.4.2 Range:

The range is the difference between the largest and the smallest value in a set of values. Maximum and minimum value recorded for each character is presented in the result.

3.1.4.3 Variance:

Variance is the average squared deviation from the sample mean. It was calculated by using following formula:

$$S^2 = (\sum (x_i - \bar{x})^2) / (n - 1)$$

Where,

S^2 = Sample variance

$\bar{x}$ = Sample mean

x_i = i^{th} element from the sample

n = Number of elements in the sample

Genotypic and Phenotypic variances estimated using the following formulae

Phenotypic variance (σ^2_p) = Variances of families

Genotypic variance (σ^2_g) = Variances of families – mean of parental variance

3.1.4.4 Standard deviation (SD)

Standard deviation is the square root of the variance. It was calculated as

$$S = \sqrt{[\Sigma (x_i - \bar{x})^2 / (n - 1)]}$$

3.1.4.5 Standard error (SE)

$$SE = SD / \sqrt{n}$$

3.1.4.6 Coefficient of variance (CV)

$$CV = \frac{SD}{\text{Mean}} \times 100$$

The components namely phenotypic variance (σ^2_p) and genotypic variance (σ^2_g) were used for estimation of phenotypic and genotypic coefficient of variation following the method suggested by Burton and Dewane (1953).

i) Phenotypic coefficient of variation (PCV)

$$PCV = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100$$

Where,

σ^2_p = Phenotypic variance

$\bar{X}$ = General mean of character

ii) Genotypic coefficient of variation (GCV)

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$$

Where,

σ^2_g = Genotypic variance

$\bar{X}$ = General mean of character

Categorization of the range of variation was effected as proposed by Sivasubramanian and Menon (1973).

< 10% - Low

10-20% - Moderate

> 20% - High

3.1.4.7 Estimation of heritability percentage

Heritability percentage in broad sense was estimated for various characters as per the formulae suggested by Hanson *et al.* (1956).

$$h^2 \text{ (bs)} = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

As suggested by Robinson *et al.* (1949) heritability in broad sense estimates were categorized as,

0-30% – Low

30-60% – Moderate

60% and above – High

3.1.4.8 Estimation of genetic advance

The genetic advance was calculated in percent by the formulae suggested by Johnson *et al.* (1955).

$$\text{Genetic Advance (GA)} = \sigma^2_g / \sigma^2_p \times \sigma_p \times k$$

Or

$$\text{GA} = k \times h^2 \text{ (bs)} \times \sigma_p$$

$$\text{GA as percentage to mean} = \frac{\text{GA}}{\bar{X}} \times 100$$

Where,

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

σ_p = Phenotypic standard deviation

K = Selection differential *i.e.* 2.06 at 5% selection intensity

h^2 (bs) = Heritability broad sense

$\bar{X}$ = Mean of character

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et al.* (1955).

< 10% - Low

10-20% - Moderate

> 20% - High

3.1.4.9 Correlation analysis

Correlation coefficient among all possible character combination at phenotypic level (r_p) was estimated (Al-Jibouri *et al.*, 1958).

$$\text{Phenotypic correlation, } r_{xy}(P) = \frac{\text{Cov}_{xy}(P)}{\sqrt{V_x(P) \times V_y(P)}}$$

Where,

$\text{Cov}_{xy}(P)$ = Phenotypic correlation between 'X' and 'Y' characters

$V_x(P)$ = Phenotypic variance of character 'X'

$V_y(P)$ = Phenotypic variance of character 'Y'

The test of significance for association between characters was done by comparing table "t" values at n-2 degree of freedom for phenotypic correlation with estimated values and correlation was performed by INDOSTAT software.

3.1.4.10 Path coefficient analysis

Path coefficient analysis suggested by Wright (1921), Dewey and Lu (1959) was carried out to know the direct and indirect effect of the morphological traits on plant yield. The following set of simultaneous equation was formed and solved for estimating various direct and indirect effects.

$$r_{1y} = a + r_{12}b + r_{13}c + \dots + r_{1i}$$

$$r_{2y} = r_{21}a + b + r_{23}c + \dots + r_{2i}$$

$$r_{3y} = r_{31}a + r_{32}b + c + \dots + r_{3i}$$

$$r_{iy} = r_{i1}a + r_{i2}b + r_{i3}c + \dots + r_{ii}$$

Where,

r_{1y} to r_{iy} = correlation coefficient between casual factors 1 to I with dependent Character y.

r_{12} to r_{ii} = correlation coefficient among casual factors

a, b, c,.....i = Direct effects of characters 'a' to 'I' with 'y' as the dependent Character

Residual effect was calculated as follows

$$\text{Residual effect (R)} = 1 - \sqrt{a^2 + b^2 + c^2 + \dots + i^2 + 2ab r_{12} + 2ac r_{13} + \dots}$$

Path coefficient analysis was performed by the INDOSTAT software.

3.1.4.11 Third and fourth degree statistics based genetics.

The third degree statistics(skewness)and fourth degree statistics (kurtosis) were estimated (Snedecor and Cochran, 1994) using 'SPSS' software program to understand the nature of genetic control of target traits. Genetic expectations of skewness ($-\frac{3}{4}d^2h$) reveal the nature of genetic control of the traits (Fisher *et al.* 1932). The parameters 'd' represents additive gene effects. Kurtosis indicates the relative number of genes controlling the traits (Robson. 1956).

Table 1: List of M₃ mutants families of Bambara groundnut used for variability studies

Sl. no	Mutants	Sl. no	Mutants	Sl. no	Mutants
1	SB42-M ₃ -F ₁ P ₁	22	SB42-M ₃ -F ₇ P ₁	43	SB42-M ₃ F ₁₃ P ₃
2	SB42-M ₃ -F ₁ P ₃	23	SB42-M ₃ -F ₇ P ₃	44	SB42-M ₃ -F ₁₄ P ₁
3	SB42-M ₃ -F ₁ P ₅	24	SB42-M ₃ -F ₇ P ₄	45	SB42-M ₃ F ₁₄ P ₆
4	SB42-M ₃ -F ₂ P ₁	25	SB42-M ₃ -F ₇ P ₆	46	SB42-M ₃ -F ₁₅ P ₁
5	SB42-M ₃ -F ₂ P ₂	26	SB42-M ₃ -F ₇ P ₈	47	SB42-M ₃ -F ₁₆ P ₁
6	SB42-M ₃ -F ₂ P ₃	27	SB42-M ₃ -F ₈ P ₁	48	SB42-M ₃ F ₁₇ P ₁
7	SB42-M ₃ -F ₃ P ₁	28	SB42-M ₃ -F ₈ P ₃	49	SB42-M ₃ -F ₁₈ P ₁
8	SB42-M ₃ -F ₃ P ₄	29	SB42-M ₃ -F ₈ P ₇	50	SB42-M ₃ -F ₁₈ P ₄
9	SB42-M ₃ -F ₃ P ₅	30	SB42-M ₃ F ₈ P ₈	51	SB42-M ₃ -F ₁₈ P ₆
10	SB42-M ₃ -F ₃ P ₇	31	SB42-M ₃ -F ₉ P ₂	52	SB42-M ₃ -F ₁₉ P ₁
11	SB42-M ₃ -F ₃ P ₁₆	32	SB42-M ₃ -F ₉ P ₃	53	SB42-M ₃ -F ₁₉ P ₃
12	SB42-M ₃ -F ₄ P ₁	33	SB42-M ₃ -F ₉ P ₄	54	SB42-M ₃ -F ₂₁ P ₂
13	SB42-M ₃ -F ₄ P ₂	34	SB42-M ₃ -F ₉ P ₇	55	SB42-M ₃ -F ₂₂ P ₁
14	SB42-M ₃ -F ₄ P ₄	35	SB42-M ₃ -F ₉ P ₈	56	SB42-M ₃ -F ₂₃ P ₁
15	SB42-M ₃ -F ₅ P ₁	36	SB42-M ₃ F ₁₀ P ₁	57	SB42-M ₃ -F ₂₃ P ₈
16	SB42-M ₃ -F ₅ P ₃	37	SB42-M ₃ -F ₁₀ P ₂	58	SB42-M ₃ -F ₂₄ P ₂
17	SB42-M ₃ -F ₅ P ₅	38	SB42-M ₃ -F ₁₀ P ₅	59	SB42-M ₃ -F ₂₆ P ₁
18	SB42-M ₃ -F ₆ P ₁	39	SB42-M ₃ -F ₁₁ P ₁	60	SB42-M ₃ -F ₂₇ P ₁
19	SB42-M ₃ -F ₆ P ₂	40	SB42-M ₃ -F ₁₁ P ₂	61	SB42-M ₃ -F ₂₈ P ₁
20	SB42-M ₃ F ₆ P ₃	41	SB42-M ₃ -F ₁₂ P ₁	62	SB42-M ₃ -F ₂₉ P ₂
21	SB42-M ₃ F ₆ P ₁₀	42	SB42-M ₃ -F ₁₃ P ₂	63	SB42-M ₃ -F ₃₀ P ₃

3.2 Experiment II– Screening for yellow mosaic virus disease in M₆ generation using SSR Markers

3.2.1 Experimental materials

Bambara groundnut variety ‘SB-42’ was used for present investigation. The present experiment-II involved seeds of M₆ generation developed using LD₅₀ (200Gy) of gamma rays (Vijaykumar. 2011 and Smita 2012).

3.2.2 Method

Seeds of M₆ generation were sown in plant to progeny row in a single replication along with parent (SB- 42) with spacing of 30 cm between rows and 10 cm within a row at K-block, GKVK, Bengaluru. N: P: K fertilizers dose of 25:75:38 kg per hectare was applied and the field was irrigated once in a week. Suitable crop protection measures were taken during the period of the crop growth.

3.2.3 DNA isolation

The genomic DNA was isolated using leaf sample of thirty days old seedlings. DNA was isolated as per the modified Cetyl trimethyl ammonium bromide (CTAB) method (Dellaporta *et al.*, 1983). The step-wise methodology detail is given below.

3.2.3.1 Protocol

Leaf samples of fresh juvenile leaves from the seedling of M₆ generation were harvested 28-30 days after sowing and kept in -20° c till the further extraction steps.



The fresh leaves were vigorously rinsed in double-distilled water to remove particles on leaf surface. These were sterilised with 70% ethanol.



Leaves were cut into small pieces with the help of sterile scissors and transferred to mortar. The leaf tissue were frozen in liquid nitrogen and ground into fine powder.



The fine powder (1 g) was allowed to thaw in the presence of 1 ml of pre-heated CTAB extraction buffer (Annexure I) in polypropylene centrifuge tubes and incubated for 45 min at 65°C in water bath with intermittent shaking.



The tubes were removed from the water bath and allowed to cool at room temperature. Equal volume of chloroform: iso-amyl alcohol mixture (24:1) was added and centrifuged at 4000 rpm for 20 min at 4°C.



The clear aqueous phase was transferred to new tube and added equal volume of phenol: chloroform (1:1) centrifuged at 4000 rpm for 20 min and repeat this step again.



Clear aqueous phase was transferred to new tube and 1/3rd volume of ice cold iso-propanol and 3 M sodium acetate added and gently mixed by inversion. Then, it was again centrifuged at 4000 rpm for 20 min at 4°C to pellet the DNA and supernatant was discarded. In this step thread like DNA was observed.



The DNA pellet was washed with 70% alcohol. After washing DNA pellet was air dried.

DNA pellet was dissolved in 100-200 µL of 1X TE buffer (pH 8.0) and stored at 4°C.

3.2.4 Quantification of DNA using Nano Drop

DNA purity and quantity was determined using Nano DropTM spectrophotometer. The ratios between the reading at 260nm and 280nm provide an estimate of DNA purity. Pure DNA samples will have a ratio of 1.8. One µL of DNA isolated from Bambara groundnut leaves were fed to the sample pedestal and the absorbance at 260 and 280nm were recorded against a buffer blank. A 260/280 nm ratio for all the samples was calculated to check the purity. DNA was quantified using the formula and further all the samples were diluted to a final concentration of 10 - 20 ng/µL.

$$\mu\text{g ds DNA}/\mu\text{L} = (A_{260} * 40)/2$$

3.2.5 Qualitative analysis of DNA

Agarose gel electrophoresis was used to separate the DNA based on size. Genomic DNA was electrophoresed in 0.8 % agarose. Details are as follows.

0.8g of agarose was weighed and taken in a clean 100ml conical flask. To this 100ml of 1x TE buffer (0.89M Tris base, 0.02MEDTA, pH = 8) was added.



Agarose was dissolved by heating. After agarose completely melted, it was cooled to 60°C and 3µL of ethidium bromide was added (10mg/ml) and mixed.

The ends of gel casting trays were sealed with tapes. Agarose was poured; comb was inserted and allowed to solidify.



After solidification the tape on either side was removed and the gel was immersed in electrophoresis tray having 1x TE buffer. Then the comb was removed.



Three µl of DNA samples and 2 µl loading dye was mixed well and loaded into the well.



Electrophoresis was carried out at 60V until the bromophenol blue dye migrated to the two-third of the gel. The gel tray was removed and the gel was observed under UV transilluminator and documented using Bio-Rad Gel Doc system.

Table 2: List of M₆ mutant lines of Bambara groundnut selected for molecular screening for yellow mosaic virus disease resistance

Sl. no	Mutants	Sl. no	Mutants	Sl. no	Mutants
1	M ₆ F ₁ P ₇	19	M ₆ F ₂₁ P ₂	38	M ₆ F ₄₁ P ₆
2	M ₆ F ₂ P ₇	20	M ₆ F ₂₁ P ₃	39	M ₆ F ₄₂ P ₂
3	M ₆ F ₃ P ₃	21	M ₆ F ₂₂ P ₂	40	M ₆ F ₄₃ P ₄
4	M ₆ F ₄ P ₆	22	M ₆ F ₂₃ P ₁	41	M ₆ F ₄₂ P ₂
5	M ₆ F ₅ P ₃	23	M ₆ F ₂₃ P ₁₀	42	M ₆ F ₄₄ P ₅
6	M ₆ F ₅ P ₄	24	M ₆ F ₂₄ P ₃	43	M ₆ F ₄₄ P ₈
7	M ₆ F ₇ P ₁	25	M ₆ F ₂₅ P ₂	44	M ₆ F ₄₅ P ₄
8	M ₆ F ₈ P ₂	26	M ₆ F ₂₆ P ₄	45	M ₆ F ₄₆ P ₃
9	M ₆ F ₉ P ₅	27	M ₆ F ₂₇ P ₄	46	M ₆ F ₄₈ P ₁
10	M ₆ F ₁₁ P ₅	28	M ₆ F ₂₉ P ₂	47	M ₆ F ₄₉ P ₂
11	M ₆ F ₁₂ P ₅	29	M ₆ F ₃₀ P ₃	48	M ₆ F ₅₃ P ₁
12	M ₆ F ₁₃ P ₃	30	M ₆ F ₃₁ P ₃	49	M ₆ F ₅₀ P ₁
13	M ₆ F ₁₄ P ₅	31	M ₆ F ₃₂ P ₁	50	M ₆ F ₅₀ P ₅
14	M ₆ F ₁₆ P ₁	32	M ₆ F ₃₄ P ₅	51	M ₆ F ₅₁ P ₂
15	M ₆ F ₁₇ P ₂	34	M ₆ F ₃₅ P ₂	52	M ₆ F ₅₇ P ₅
16	M ₆ F ₁₈ P ₆	35	M ₆ F ₃₇ P ₃	53	M ₆ F ₅₈ P ₄
17	M ₆ F ₁₉ P ₁	36	M ₆ F ₃₉ P ₁	54	M ₆ F ₆₀ P ₄
18	M ₆ F ₂₀ P ₉	37	M ₆ F ₄₁ P ₂	55	M ₆ F ₆₁ P ₂

3.2.6 Designing of Primer

Since yellow mosaic virus resistance gene sequence has not been reported in *Vigna subterranea*. Heterologous probes designed from its phylogenetically related species choosen for the present investigation. *Vigna mungo* resistance protein (VMYR-1) gene(table 3a) of the GenBank accession: AY297425.2, which is 445 bp linear DNA reported to be Yellow mosaic virus resistance linked DNA marker sequence in *Vigna mungo* (Basak *et al.* 2004) sequence was collected from NCBI (National Centre for Biotechnology Information) site.

The sequence was further analysed using Bioinformatics tool Basic Local Alignment Search Tool (BLAST), after this sequence was found to be present in other species of *Vigna* and is responsible for yellow mosaic virus disease resistance (Table 3b). Hence this gene sequence was considered as a template sequence to design the forward and reverse primers for PCR amplification and genome specific primers were designed using primer-Blast, synthesized from Chromous Biotech, Bangalore, India.

Table 3a: Complete sequence of *Vigna mungo* resistance protein (VMYR-1)

```
AGTTTATAATTCGATTGCTGATGATTTTGATAGTTCATGTTTTCTTCAAAATGT
AAGAGAAGAATCAAATAAACATGGGTAAAACACCTCCAGAGCCTCCTTCTT
TCAGAAATACTTGGAGAGAACGACATCATCTTAGCAAGTGTGCAAAGAGGAA
TTTCAGTGATACAACAAAGGCTTGGTAGAAAGAAAGTTCTCTTGATTCTAGAT
GATGTTGACAACAGAAAGCAGTTACAAGCATTTGCTGGAAGATCTGATTGGT
TTGGTCCCGGCAGCAGAGTTATCGTTACCACTCGGGACGAACAGCTGCTAAA
ATCTCATGAGATTGAAAGAACTTATGAGGTGGAGGAATTGAATGATAATGAT
TCTCTTCAATTGCTTATATGGAATGCTTTCAAAGGGAAAAAGTTGATCCAAG
ATACGAGGACGTCTTGAATCGTAGT
```

Table 3: BLAST result for Yellow Mosaic Virus sequence across the *Vigna species*

Description	Total Score	Query cover	E value	Max identity	Accession
<i>Vigna mungo</i> disease resistance protein (VMYR-1) gene, partial cds	822	100%	0.0	100%	AY297425.2
<i>Vigna radiate</i> viral resistance candidate (MYR-1) gene, partial cds	673	87%	0.0	98%	AY301990.1
<i>Vigna mungo</i> disease resistance protein (MYR-3) mRNA, partial cds	667	100%	0.0	96%	EF446378.1
<i>Vigna mungo</i> disease resistance protein (VMYR-2) gene, partial cds	560	88%	2e-160	92%	AY301991.1
<i>Vigna unguiculata</i> clone KIND12 disease resistance protein homolog gene, partial cds	307	62%	3e-84	87%	AF141012.1
<i>Vigna unguiculata</i> clone KINE12 disease resistance protein homolog gene, partial cds	303	62%	4e-83	86%	AF141013.1
<i>Vigna vexillata</i> disease resistance protein homolog gene sequence	198	63%	2e-51	79%	AF141014.1

Table 4. Primer sequences obtained for yellow mosaic virus resistant sequence

Sl. no	Sequences	Length	Start	Stop	Tm (°C)	G+C (%)	Product length
1.	Forward: ATGATAACTCTGCTGCCGGG	20	101	120	59.89	55	202
	Reverse: ACCTCCAGACCGTCCTTCTT	20	302	283	60.18	55	
2.	Forward: GATAACTCTGCTGCCGGGAC	20	103	122	60.53	60	203
	Reverse: AACACCTCCAGACCGTCCTT	20	305	286	60.76	55	
3.	Forward: ACCTCCAGAGCCTCCTTCTT	20	116	135	59.96	55	192
	Reverse: CCGGGACCAAACCAATCAA	20	302	282	59.96	55	
4.	Forward: CTGTTTCGTCCCGAGTGGTAA	20	82	101	59.40	55	225
	Reverse: AAACACCTCCAGACCGTCCT	20	306	287	60.76	55	
5.	Forward: TTTAGCAGCTGTTCGTCCCG	20	74	93	60.67	55	230
	Reverse: CACCTCCAGACCGTCCTTCT	20	303	284	60.90	60	

3.6.5.2 SSR Reaction mixture

The SSR reaction mixture consisted of 2µL of template DNA, 0.5 µL of each of forward and reverse primer, 2X PCR master mix containing *Taq* DNA polymerase, assay buffer and dNTPs in a volume of 10 µl. The PCR mixture for one reaction is given below.

SSR reaction mixture	1x
Template DNA (10 ng)	2.0 µl
Primer mix (forward and reverse)	1.0µl
dNTP mix (1mM)	1.0 µl
<i>Taq</i> buffer (10X)	1.0 µl
<i>Taq</i> polymerase (5U/µl)	0.3 µl
Sterile water	4.7 µl
Total	10.0 µl

3.6.5.3 Amplification condition

Eppendorf PCR programme me was used to amplify the DNA fragments. The primers used in the study were with the different annealing temperature. The details of the programme used are given below.

Steps	Reactions	Temp (⁰ C)	Duration
1 :	Initial denaturation	94	4 min
2 :	Denaturation	94	1 min
3 :	Annealing	60	45 sec
4 :	Extension	72	1 min
5 :	Final Extension	72	8 min
6 :	Hold	4	30 min

3.6.5.4 Gel electrophoresis

The product were analyzed using 2% agarose gel in a volume of electrophoresis buffer (1x TBE) sufficient for constructing a gel. Ethidium bromide was added at concentration of 0.5 µg/ml of gel. The gel was allowed to set fully before removing the comb and loading the sample. Three µl of loading dye was added to 10 µl of PCR products and mixed well before loading into the wells. Care was taken to prevent mixing of samples between the wells. A current of 60V for 45min and after the electrophoresis, the DNA banding pattern was viewed under UV trans-illuminator (BIO-RAD gel documentation system) and documented.



Plate 1: Yellow mosaic virus disease susceptible plant



Plate 2: Yellow mosaic virus disease resistant plant

IV EXPERIMENTAL RESULTS

The present investigation was conducted during *kharif* 2014 at K block, Department of Genetics and Plant Breeding, GKVK, UAS, Bengaluru and the experimental results are presented under following subtitles,

4.1 Comparison of mean performance of mutants with the parent

4.2 Genetic variability parameters in M₃ generation

4.3 Skewness and Kurtosis

4.4 Correlation coefficients

4.5 Path coefficient analysis

4.6 Molecular screening for yellow mosaic virus disease

4.1 Comparison of mean performance mutants with the parent

Mean, Shift in mean, and *per cent* mean of parent for different quantitative traits like days to flowering, plant height, number of branches per plant, plant spread, number of pods per plant, pod yield per plant, seed yield per plant, shelling percentage, 100-seed weight and plant dry weight of parent population (SB-42) and M₃ generation are presented in table 5.

4.1.1 Days to flowering

It was observed that there was significant decrease in the mean value of mutants for days to flowering in M₃ generation (53 days) compared to parent (54 days). The mean of the M₃ generation shifted towards negative direction (-1.02) and *per cent* mean over parent for days to flowering was -1.89 % and created significant variation in mutant population.

4.1.2 Plant height (cm)

It was observed that there was a significant decrease in the mean value of mutant for plant height in M₃ generation (18.24 cm) compared to parent (22.21 cm). The mean of the M₃ generation shifted towards negative direction (-3.97) and *per cent* mean over parent for plant height was -17.89 % and created significant variation in mutant population.

Table 5: Comparison of parental trait mean with M₃ population derived from SB-42

Sl. no.	Characters	Mean		Shift in mean	P (T ≤ t)	<i>per cent mean of parent</i>
		Parent	Mutants			
1	Days to flowering	54.05	53.03	-1.02	0.00	-1.88
2	Plant height (cm)	22.21	18.24	-3.97	0.00	-17.89
3	Number of branches	32.70	37.31	4.61	0.00	14.09
4	Plant spread (cm)	24.49	26.27	1.78	0.00	7.28
5	Number of pods	33.85	36.86	3.01	0.00	8.88
6	Pod yield (gm)	23.84	26.14	2.30	0.00	9.64
7	Seed yield (gm)	15.24	17.06	1.83	0.00	11.99
8	Shelling percentage (%)	63.93	65.67	1.73	0.05	2.71
9	100 seed weight (gm)	44.82	46.26	1.44	0.18	3.21
10	Plant dry weight (gm)	14.47	17.12	2.66	0.00	18.38

P < 0.05 significance at 5% level of significance

P < 0.01 significance at 1% level of significance

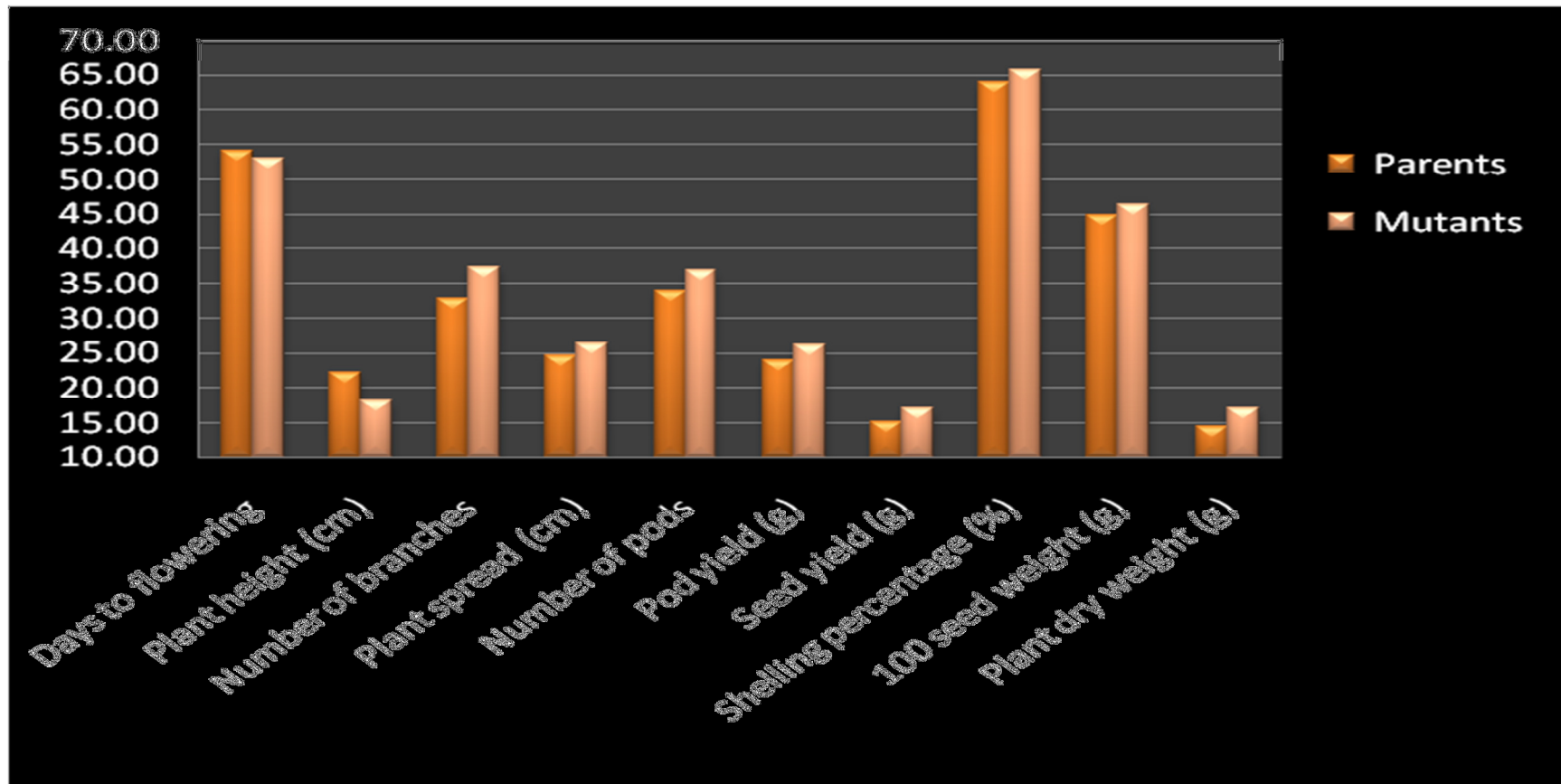


Fig. 1 Comparison of mean performance of mutants with the parent for ten quantitative characters

4.1.3 Number of branches per plant

It was noticed that there was high significant increase in the mean value of mutants for number of branches per plant in M₃ generation (37.31) compared to parent (32.70). The mean of the M₃ generation shifted towards positive direction (4.61) and *per cent* mean over parent for number of branches per plant was 14.09 % and significant variation in the population.

4.1.4 Plant spread (cm)

It was observed that there was a high significant increase in the mean value of mutants for plant spread in M₃ generation (26.27 cm) compared to parent (24.49 cm). The mean of the M₃ generation shifted towards positive direction (1.78 cm) and *per cent* mean over parent for plant spread was 7.28 % and there was significant variation in the population.

4.1.5 Number of pods per plant

It was found that there was a high significant increase in the mean value of mutants for number of pods per plant in M₃ generation (36.86) compared to parent (33.85). The mean of the M₃ generation shifted towards positive direction (3.01) and *per cent* mean over parent for number of pods per plant was 8.88 % and there observed the significant variation in the population.

4.1.6 Pod yield per plant (g)

It was found that there was a high significant increase in the mean value of mutants for pod yield per plant per plant in M₃ generation (26.14 g) compared to parent (23.84 g). The mean of the M₃ generation shifted towards positive direction (2.30) and *per cent* mean over parent for pod yield per plant per plant was 9.64 % and significant increase in the variation.

4.1.7 Seed yield per plant (g)

It was found that, high significant increase in the mean value of mutants for seed yield per plant in M₃ generation (17.06 g) compared to parent (15.24 g). The mean of the M₃ generation shifted towards positive direction (1.83 g) and *per cent* mean over parent for seed yield per plant was 11.99 % and significant increase in the variation.

4.1.8 Shelling percentage (%)

It was observed that there was significant increase in the mean value of mutants for shelling percentage in M₃ generation (65.67) compared to parent (63.93). The mean of the M₃ generation shifted towards positive direction (1.73) and *per cent* mean over parent for shelling percentage was 2.71 % and significant increase in the variation for this trait.

4.1.9 Hundred seed weight (g)

It was observed that there was non-significant increase in the mean value of mutants for hundred seed weight in M₃ generation (46.26 g) compared to parent (44.82 g). The mean of the M₃ generation shifted towards positive direction (1.44 g) and *per cent* mean over parent for hundred seed weight was 3.21 % and significance increase in the variation for this trait.

4.1.10 Plant dry weight (g)

It was found that there was a high significant increase in the mean values for plant dry weight in M₃ generation (17.12 g) compared to parent (14.47 g). The mean of the M₃ generation shifted towards positive direction (2.66 g) and *per cent* mean over parent for plant dry weight was 18.38 % and observed the significance variation in the population for this trait.

4.2 Genetic variability parameters in M₃ generation

Mean, range, phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), heritability and genetic advance as *per cent* of mean for ten quantitative characters viz., days to flowering, plant height, number of branches per plant, plant spread, pod yield per plant, seed yield per plant, , number of pods per plant, shelling percentage, 100-seed weight and plant dry weight in M₃ generation of Bambara groundnut are presented in Table 6 and 7 (7a, 7b, 7c, 7d).

4.2.1 Days to flowering

Overall mean for days to flowering for families was 53.03 days and wide range of variation from as early as 50 days (family 14) to as late as 55.20 days (family 61). Wide range of days to flowering was taken by the family 60 (49-56 days) and family 42 (4.04) showed maximum variance among all the families.

Genotypic and phenotypic coefficients of variation were 2.42 and 2.67 per cent respectively. This trait had high heritability (82.03 %) with low expected genetic advance as percent mean (4.52).

4.2.2 Plant height (cm)

Plant height ranged from 12.85cm (Family 28) to 23.25 cm (family 63) with an overall mean of 18.24 cm. Wide range was observed in family 35 (11-24 cm) with the higher variance of 16.90 among the all families.

Genotypic and phenotypic coefficients of variation were 9.92 and 11.16 per cent respectively. This trait showed high heritability (79.00 %) with moderate *per cent* mean of genetic advance (18.16).

Table 6: Mean, range, coefficient of variation, heritability and genetic advance as *per cent* of mean for ten quantitative characters in M₃ generation of Bambara groundnut

Sl. no.	Characters	Mean $\pm$ SE	Range		Co-efficient of variability		Heritability (broad sense) (%)	GA as % Mean
			Min	Max	Phenotypic (%)	Genotypic (%)		
1	Days to flowering	53.03 $\pm$ 0.18	50.00	55.20	2.67	2.42	82.03	4.52
2	Plant height (cm)	18.24 $\pm$ 0.26	12.85	23.25	11.16	9.92	79.00	18.16
3	Number of branches	37.31 $\pm$ 1.15	25.10	61.40	24.53	24.22	97.52	49.28
4	Plant spread (cm)	26.27 $\pm$ 0.42	15.95	35.51	12.65	12.10	91.61	23.86
5	Number of pods	36.86 $\pm$ 0.71	18.90	52.30	15.28	14.75	93.25	29.35
6	Pod yield (g)	26.14 $\pm$ 0.46	11.96	37.51	14.09	13.27	88.74	25.75
7	Seed yield (g)	17.06 $\pm$ 0.28	9.12	24.26	12.96	12.11	87.28	23.31
8	Shelling percentage (%)	65.67 $\pm$ 0.44	56.64	76.87	5.32	4.44	69.59	7.62
9	100 seed weight (g)	46.26 $\pm$ 0.51	38.23	56.55	8.70	7.65	77.42	13.87
10	Plant dry weight (g)	17.12 $\pm$.031	8.34	21.93	14.38	11.71	66.31	19.64

Table 7a: Mean, range and variance for days to flowering, plant height and branches per plant in mutant families of M₃ generation of Bambara groundnut

	Days to flowering				Plant height (cm)				Number branches per plant			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
1	52.20	54.00	51.00	0.84	18.80	22.50	14.00	9.01	31.30	34.00	29.00	3.34
2	53.80	55.00	52.00	1.07	18.65	23.00	15.00	4.78	32.50	37.00	29.00	7.39
3	50.60	53.00	48.00	2.93	17.95	21.00	14.50	4.02	27.70	35.00	22.00	13.34
4	54.20	55.00	53.00	0.40	18.95	25.00	13.50	9.75	33.70	39.00	26.00	12.46
5	54.60	56.00	53.00	0.93	16.55	21.00	12.00	10.80	32.40	37.00	29.00	8.49
6	55.10	54.00	51.00	0.90	16.40	21.00	12.00	9.66	33.30	39.00	29.00	14.23
7	53.30	55.00	51.00	1.34	19.70	23.00	16.50	5.96	38.50	42.00	34.00	7.83
8	52.10	55.00	50.00	1.88	18.25	22.00	15.00	6.96	30.60	38.00	26.00	15.38
9	52.20	54.00	49.00	2.40	19.15	25.00	15.00	8.67	38.90	45.00	34.00	12.10
10	51.30	53.00	49.00	1.79	20.45	25.00	17.00	5.58	44.80	50.00	42.00	6.84
11	51.90	54.00	50.00	1.66	18.40	23.00	14.00	10.71	25.70	29.00	23.00	3.57
12	50.80	53.00	49.00	2.62	18.25	25.00	13.00	13.40	46.30	53.00	43.00	8.46
13	52.40	54.00	50.00	2.04	17.05	21.50	11.50	14.97	34.60	39.00	32.00	4.71
14	50.00	52.00	49.00	0.89	20.50	24.00	16.00	6.56	44.90	47.00	42.00	2.77

	Days to flowering				Plant height (cm)				Number branches per plant			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
15	51.70	54.00	49.00	2.23	18.10	24.00	12.00	12.71	32.10	38.00	29.00	6.32
16	52.50	55.00	50.00	2.50	15.90	22.00	11.00	12.60	54.20	59.00	52.00	4.18
17	52.30	55.00	50.00	2.23	19.70	25.00	15.00	9.34	32.80	38.00	29.00	9.96
18	54.60	56.00	54.00	0.49	20.85	26.00	17.50	7.34	29.50	35.00	21.00	16.06
19	52.90	54.00	52.00	0.77	14.95	19.00	10.50	7.52	37.80	42.00	32.00	12.62
20	50.20	52.00	49.00	1.51	18.65	23.00	14.00	6.67	34.10	38.00	31.00	4.10
21	53.20	55.00	51.00	1.73	18.45	22.00	13.00	7.14	28.10	31.00	25.00	2.99
22	52.70	54.00	51.00	0.90	16.85	21.00	12.00	6.78	45.90	49.00	43.00	3.66
23	53.40	55.00	52.00	1.38	18.10	24.00	14.00	11.27	52.60	56.00	50.00	2.93
24	53.90	56.00	51.00	2.32	16.55	21.00	13.00	6.02	25.40	28.00	24.00	1.38
25	54.40	56.00	53.00	1.16	18.10	23.00	14.00	11.82	42.90	46.00	41.00	3.66
26	53.20	55.00	52.00	1.07	15.80	19.00	12.50	5.23	46.00	51.00	42.00	8.22
27	54.50	56.00	54.00	0.50	17.25	23.00	13.00	6.90	28.60	32.00	25.00	4.49
28	52.00	54.00	50.00	2.22	12.85	18.00	8.50	9.89	33.70	36.00	31.00	2.68
29	54.00	55.00	52.00	1.11	13.80	21.00	10.00	8.46	27.30	32.00	23.00	10.23
30	53.00	54.00	50.00	1.60	13.83	17.00	10.00	6.12	34.80	38.00	31.00	3.73
31	52.80	54.00	51.00	1.07	17.15	23.00	12.00	10.67	45.70	49.00	42.00	5.12

	Days to flowering				Plant height (cm)				Number branches per plant			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
32	52.70	54.00	51.00	1.34	17.80	24.00	13.00	11.07	52.20	56.00	51.00	2.40
33	53.00	55.00	50.00	2.00	17.50	21.00	10.00	11.61	29.00	33.00	24.00	9.78
34	50.10	52.00	48.00	1.43	18.00	21.00	13.00	5.56	42.00	46.00	39.00	3.78
35	53.20	54.00	52.00	0.62	18.30	24.00	11.00	16.90	32.80	38.00	26.00	13.51
36	53.80	56.00	52.00	1.51	15.10	19.00	10.00	9.88	40.30	42.00	38.00	1.79
37	55.00	56.00	53.00	0.72	20.90	25.00	13.00	12.54	47.10	52.00	42.00	10.99
38	53.90	56.00	52.00	1.21	16.70	21.00	13.00	5.79	37.50	41.00	34.00	6.50
39	53.70	55.00	52.00	0.90	17.70	21.00	15.00	3.57	32.40	38.00	29.00	8.04
40	54.10	57.00	52.00	2.32	20.20	23.00	16.00	4.34	34.70	42.00	31.00	10.01
41	53.50	55.00	52.00	0.72	19.60	23.00	14.00	7.82	39.10	42.00	36.00	4.10
42	50.20	55.00	49.00	4.04	18.35	24.00	15.00	6.45	39.50	44.00	35.00	7.39
43	53.80	56.00	52.00	1.51	17.55	21.00	14.00	4.80	51.70	54.00	48.00	3.12
44	53.10	55.00	52.00	1.21	18.75	23.00	15.00	6.18	36.00	42.00	32.00	8.89
45	50.10	53.00	48.00	1.88	17.40	21.00	14.00	6.21	32.60	38.00	29.00	7.60
46	55.05	56.00	53.00	0.72	16.15	21.00	10.00	10.00	28.10	32.00	24.00	7.88
47	53.50	55.00	52.00	1.17	16.70	21.00	12.00	8.01	26.80	32.00	24.00	7.73
48	54.12	55.00	50.00	1.96	19.40	24.00	14.00	8.49	31.10	34.00	29.00	2.54

	Days to flowering				Plant height (cm)				Number branches per plant			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
49	54.80	56.00	53.00	1.07	19.45	26.00	14.00	11.30	25.10	29.00	20.00	9.21
50	53.00	55.00	51.00	1.61	16.20	19.00	13.00	4.62	27.90	32.00	23.00	7.66
51	54.40	56.00	52.00	1.16	18.90	23.00	15.00	7.60	37.20	43.00	32.00	9.51
52	52.80	55.00	51.00	1.29	19.90	24.00	14.00	10.99	32.90	40.00	30.00	9.66
53	53.10	56.00	52.00	1.51	18.25	21.00	15.00	4.40	34.40	41.00	30.00	12.27
54	52.50	53.00	49.00	1.56	18.15	21.00	13.50	5.95	33.30	36.00	31.00	2.46
55	50.60	53.00	50.00	1.07	18.00	24.00	12.50	9.50	30.10	36.00	27.00	6.77
56	53.50	56.00	51.00	1.88	21.65	27.00	16.50	11.67	31.80	38.00	28.00	11.29
57	53.10	54.00	50.00	1.43	17.60	23.00	15.00	8.04	27.60	32.00	25.00	5.60
58	55.10	55.00	53.00	0.77	20.00	25.00	15.00	7.56	30.40	33.00	28.00	3.82
59	54.50	56.00	53.00	0.72	19.15	22.50	16.50	3.61	61.40	67.00	56.00	7.82
60	50.80	56.00	49.00	2.68	21.65	24.50	18.00	4.11	51.90	54.00	49.00	2.32
61	55.20	56.00	54.00	0.62	21.50	25.00	18.00	5.11	54.40	56.00	52.00	1.60
62	54.50	55.00	53.00	0.68	23.20	26.50	19.50	5.96	51.50	53.00	50.00	1.17
63	53.80	55.00	53.00	0.62	23.25	27.00	18.00	7.90	60.90	64.00	58.00	4.54

Table 7b: Mean, range and variance for plant spread, number of pods per plant and pod yield per plant in mutant families of M₃ generation of Bambara groundnut

	Plant spread (cm)				Number of pods per plant				Pod yield (g)			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
1	27.75	32.50	23.50	6.13	37.30	43.00	31.00	12.23	28.90	32.10	23.10	7.38
2	25.44	28.00	22.00	4.39	32.10	38.00	27.00	10.10	22.79	24.60	19.90	2.80
3	22.41	25.00	20.10	2.79	27.60	33.00	23.00	9.82	22.93	28.00	19.70	5.70
4	21.91	23.10	19.50	1.65	39.60	43.00	35.00	7.82	29.72	34.30	20.70	16.81
5	24.58	29.00	21.00	8.33	26.60	33.00	21.00	13.82	20.82	26.00	15.90	12.13
6	28.33	32.00	26.00	4.68	40.40	46.00	36.00	10.49	28.54	32.90	23.70	10.49
7	28.80	32.50	24.00	8.23	41.40	49.00	37.00	14.04	29.06	33.10	25.30	7.80
8	27.46	31.50	24.00	5.83	33.70	39.00	27.00	10.01	24.17	31.30	18.30	17.99
9	25.70	32.50	21.00	8.57	35.80	42.00	31.00	13.29	26.59	33.90	23.10	10.91
10	29.90	33.00	27.50	2.88	41.45	49.00	37.00	15.14	27.35	33.20	24.60	5.98
11	25.55	28.50	22.00	5.30	37.10	41.00	34.00	4.99	26.48	33.10	22.40	12.72
12	24.94	28.50	22.00	4.67	36.80	42.00	31.00	10.62	26.72	29.80	18.80	9.29
13	20.30	23.50	18.00	3.07	35.00	40.00	31.00	8.89	24.83	34.10	20.60	19.39
14	30.55	34.50	26.00	8.53	39.40	44.00	33.00	13.38	28.83	32.10	22.80	10.21
15	27.45	32.00	21.50	10.19	35.50	41.00	30.00	11.83	23.90	28.70	16.80	10.45

	Plant spread (cm)				Number of pods per plant				Pod yield (g)			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
16	24.45	26.50	23.00	1.14	36.40	44.00	31.00	14.04	26.60	32.10	19.70	15.94
17	28.60	31.00	24.50	4.77	36.50	41.00	29.00	12.06	26.78	31.90	20.30	15.06
18	31.80	36.00	28.00	6.34	36.20	40.00	31.00	11.07	26.54	31.20	19.70	11.47
19	21.70	25.50	18.50	4.96	41.20	46.00	37.00	7.07	27.84	30.60	25.30	2.70
20	27.28	32.00	22.50	6.76	38.70	43.00	35.00	7.34	28.95	33.60	25.60	8.01
21	26.10	30.50	24.00	4.43	47.20	55.00	41.00	15.07	29.63	36.30	22.90	18.87
22	25.60	27.50	24.00	1.82	40.50	45.00	35.00	9.83	29.76	32.60	25.40	5.51
23	23.90	26.50	21.50	1.77	33.90	40.00	29.00	14.54	24.91	28.00	20.90	4.81
24	24.65	28.50	21.00	5.95	35.70	43.00	31.00	13.34	28.63	32.30	22.70	8.82
25	25.65	30.00	19.50	9.22	35.40	41.00	32.00	8.71	28.15	33.10	24.30	8.18
26	23.30	27.00	18.50	8.84	30.10	35.00	25.00	15.43	22.15	23.90	18.70	2.26
27	20.25	24.00	18.00	3.01	28.30	33.00	25.00	8.23	21.70	25.80	18.70	5.02
28	15.95	19.00	12.50	4.86	18.90	25.00	13.00	12.99	11.96	17.10	8.70	6.45
29	21.65	28.50	18.00	9.56	30.20	36.00	25.00	14.40	21.86	27.30	17.40	9.13
30	21.65	26.00	18.00	10.00	34.20	40.00	30.00	11.29	22.49	29.00	19.10	13.09
31	24.30	30.00	18.00	11.34	36.30	43.00	30.00	13.34	25.69	30.60	19.70	11.70
32	24.85	27.00	23.50	1.50	33.30	40.00	28.00	14.23	23.53	31.00	18.30	13.34

	Plant spread (cm)				Number of pods per plant				Pod yield (g)			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
33	25.14	28.00	21.00	6.34	29.50	36.00	25.00	9.61	20.89	27.10	17.00	12.44
34	26.45	29.00	24.50	1.69	41.20	48.00	37.00	15.29	27.57	33.40	22.70	11.13
35	25.40	28.50	22.50	3.93	39.70	44.00	36.00	5.57	27.73	31.80	23.40	6.63
36	22.80	26.00	20.50	2.90	36.30	39.00	34.00	2.68	23.99	27.90	19.10	7.45
37	22.95	28.00	20.00	5.58	44.50	50.00	41.00	5.83	28.23	33.90	25.00	6.95
38	24.70	29.00	18.00	11.57	36.20	42.00	31.00	11.96	25.94	31.00	20.90	12.78
39	26.20	31.00	21.00	8.62	34.70	41.00	30.00	14.01	24.78	30.30	19.40	12.57
40	30.55	39.00	24.00	15.69	37.90	47.00	34.00	14.32	27.00	31.30	21.20	9.55
41	28.10	33.00	21.00	13.66	41.60	49.00	37.00	15.82	30.81	39.30	26.60	14.90
42	26.60	31.00	21.00	8.71	40.10	46.00	35.00	12.99	27.76	32.30	21.80	10.12
43	25.75	31.00	21.00	11.40	32.60	38.00	28.00	11.82	21.91	27.60	18.40	10.00
44	27.90	36.00	21.00	16.54	43.70	54.00	38.00	24.23	25.91	30.10	23.00	6.69
45	24.50	29.00	21.00	7.39	34.60	40.00	27.00	17.16	23.30	27.60	16.80	14.29
46	25.60	30.00	19.00	12.71	31.40	38.00	25.00	13.82	21.67	28.70	16.90	10.53
47	25.15	31.00	20.00	13.67	34.90	42.00	29.00	18.54	22.46	29.10	16.10	15.83
48	28.65	33.00	24.00	7.56	44.60	48.00	41.00	6.93	27.82	33.10	22.70	11.52
49	28.80	34.00	23.00	9.51	41.70	47.00	37.00	8.90	25.75	31.00	20.70	7.01

	Plant spread (cm)				Number of pods per plant				Pod yield (g)			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
50	24.10	28.00	18.50	11.82	47.20	53.00	44.00	8.18	29.86	35.30	26.00	10.24
51	27.20	32.00	21.00	13.73	40.20	44.00	37.00	5.96	26.44	29.60	24.20	3.74
52	28.50	34.00	24.00	12.00	39.70	45.00	35.00	8.46	26.84	30.90	19.60	10.27
53	27.20	32.00	21.00	12.40	33.40	47.00	25.00	40.49	25.83	34.40	20.30	20.05
54	29.60	35.00	26.00	9.60	38.10	43.00	34.00	8.99	28.13	32.30	23.40	7.83
55	27.55	32.00	23.00	10.69	29.20	36.00	24.00	13.07	22.77	25.80	18.70	4.21
56	28.90	32.00	24.00	8.66	31.50	37.00	26.00	19.39	24.90	31.00	19.00	14.38
57	26.35	31.50	21.00	12.78	35.20	46.00	29.00	27.73	25.89	34.00	20.20	18.34
58	28.60	31.50	23.00	8.32	35.85	41.00	31.00	8.67	25.51	29.00	21.90	5.61
59	28.55	33.00	25.00	7.14	32.40	36.00	29.00	4.93	25.23	29.10	22.90	5.00
60	32.75	39.00	28.00	11.63	39.30	44.00	35.00	7.34	30.19	36.20	25.90	10.41
61	29.75	34.00	26.00	6.07	41.90	47.00	36.00	10.99	29.58	34.90	25.10	10.04
62	32.65	41.00	29.50	12.00	52.30	58.00	47.00	10.01	37.51	41.00	33.80	5.30
63	35.51	42.50	31.00	15.82	47.80	56.00	43.00	13.73	35.69	40.90	33.10	5.58

Table 7c: Mean, range and variance for seed yield, shelling percentage and 100 seed weight in mutant families of M₃ generation of Bambara groundnut

	Seed yield (g)				Shelling percentage (%)				100 seed weight (g)			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
1	21.04	23.00	17.00	3.52	72.86	76.98	70.36	3.96	54.93	58.29	51.26	3.92
2	16.77	18.60	14.90	1.27	73.66	76.92	69.39	6.23	52.46	58.62	42.50	29.23
3	15.36	17.90	12.90	2.02	67.08	72.46	63.93	6.41	56.55	60.00	52.13	7.20
4	18.26	20.70	13.60	4.33	61.68	65.70	59.02	4.77	46.07	51.84	36.48	18.38
5	13.91	16.80	10.80	3.73	67.12	72.50	63.45	6.15	47.81	52.86	36.41	24.20
6	17.64	19.70	14.80	3.19	61.93	69.00	58.82	8.08	43.67	48.05	40.81	8.59
7	17.59	20.10	15.80	2.27	60.66	63.51	52.83	9.23	42.64	48.05	36.74	12.74
8	15.43	19.10	11.70	6.14	64.04	67.96	60.38	6.81	41.68	47.58	34.12	12.48
9	16.88	22.40	14.70	4.71	63.55	67.36	57.24	9.83	47.24	53.33	42.16	17.17
10	17.17	20.70	16.10	1.92	62.84	65.45	60.58	2.42	41.53	44.62	38.60	5.08
11	16.30	19.90	14.00	3.63	61.70	66.23	59.50	3.56	43.81	48.54	40.00	7.20
12	16.82	19.00	13.10	2.50	63.16	69.68	59.26	7.33	45.72	48.86	42.26	4.71
13	15.14	20.10	12.00	6.53	61.04	62.67	58.25	2.45	42.71	50.00	36.45	19.39
14	17.98	21.00	15.00	3.98	62.47	67.57	56.11	12.35	43.69	45.45	41.84	1.43
15	14.96	17.30	11.70	2.50	62.90	69.64	60.28	8.73	40.60	46.18	36.45	7.76

	Seed yield (g)				Shelling percentage (%)				100 seed weight (g)			
	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
16	16.91	19.80	13.00	3.93	63.88	67.70	60.44	6.47	45.21	48.82	36.48	16.65
17	17.31	20.10	14.00	3.55	64.96	68.97	61.02	7.33	47.27	50.83	40.73	11.16
18	16.25	18.00	13.00	2.14	61.53	66.52	57.69	7.91	44.97	48.71	40.51	8.33
19	15.75	16.60	14.60	0.45	56.64	58.53	52.61	2.99	38.32	40.81	35.00	4.45
20	16.93	19.70	14.90	2.10	58.56	61.33	56.25	3.36	43.77	46.90	39.77	6.34
21	19.68	25.00	15.70	9.20	66.46	71.89	59.50	12.57	38.23	41.65	35.64	3.32
22	18.54	20.70	16.00	2.93	62.31	66.55	56.34	9.59	45.30	50.51	37.92	26.07
23	16.16	18.10	13.90	1.86	64.94	69.35	60.47	6.71	47.86	53.00	44.25	8.17
24	18.28	21.30	15.70	3.48	63.94	69.16	60.69	8.52	50.80	55.24	44.31	10.02
25	17.58	20.30	15.70	2.61	62.52	65.43	60.15	2.32	49.69	53.43	44.86	7.61
26	15.18	16.70	12.90	1.45	67.49	73.21	62.90	9.43	46.97	51.07	40.62	11.11
27	14.63	16.90	12.60	2.07	67.49	71.86	65.09	5.90	49.13	52.96	42.60	12.77
28	9.12	12.10	7.00	2.33	76.87	82.73	70.76	14.92	48.57	53.85	43.33	10.07
29	14.45	16.60	11.80	1.90	66.47	73.30	60.77	13.73	48.07	56.00	46.11	9.26
30	14.90	18.10	13.40	2.95	66.67	71.20	62.07	11.69	43.60	48.67	38.86	8.82
31	17.29	19.90	14.60	2.89	67.65	74.11	64.05	12.79	47.74	54.33	43.61	10.71
32	14.64	18.00	13.10	2.69	62.66	71.58	58.06	16.91	44.09	47.86	38.29	9.94

	Seed yield (g)				Shelling percentage (%)				100 seed weight (g)			
	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
33	14.12	16.90	11.80	2.78	68.13	72.63	61.92	19.06	47.87	51.94	41.94	7.89
34	17.18	20.70	14.70	3.23	62.51	67.84	55.34	12.95	41.78	46.22	36.89	11.09
35	18.20	20.10	16.10	1.40	65.85	74.40	61.86	13.57	45.89	50.25	42.27	6.74
36	16.03	18.70	14.00	1.83	67.08	73.30	62.41	10.29	43.56	48.82	40.00	7.54
37	18.30	22.10	16.10	3.38	64.81	68.40	61.11	4.27	41.08	45.81	36.67	7.28
38	17.47	19.70	14.90	3.32	67.68	75.23	63.55	11.19	48.30	54.72	44.47	9.14
39	16.57	18.80	14.60	2.46	67.32	75.26	62.05	13.86	46.76	51.52	43.24	6.68
40	17.97	19.90	15.60	1.59	66.91	73.58	62.24	13.82	47.63	52.43	42.34	12.98
41	20.08	25.90	17.70	6.65	65.20	69.34	61.61	5.21	48.33	55.71	42.44	20.32
42	18.17	21.30	15.40	3.05	65.64	70.64	61.01	8.02	45.40	51.05	41.71	11.77
43	14.86	18.40	12.60	4.28	67.90	72.33	64.95	5.96	45.65	53.10	38.18	21.50
44	17.68	20.90	15.70	2.80	68.34	73.48	61.29	10.42	40.62	44.47	35.63	8.62
45	17.22	20.70	12.30	6.45	74.14	79.10	67.03	13.34	49.74	57.43	45.43	16.81
46	14.66	18.00	12.10	3.65	67.89	73.42	62.72	12.52	46.76	51.29	40.29	12.29
47	15.60	18.60	12.00	6.01	69.73	74.53	63.23	9.73	44.68	51.21	38.71	19.24
48	17.87	20.60	14.70	3.76	64.37	68.40	60.42	7.14	40.00	43.78	35.85	7.72
49	16.87	19.60	14.10	2.98	65.56	68.46	61.51	5.80	40.48	47.75	35.25	11.27

	Seed yield (g)				Shelling percentage (%)				100 seed weight (g)			
Families	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance	Mean	Max.	Min.	Variance
50	18.44	21.00	15.70	3.38	61.83	66.08	59.02	5.87	39.03	42.83	35.32	6.35
51	17.73	20.90	14.30	4.04	66.95	72.57	59.09	18.27	44.05	48.60	38.29	12.01
52	17.76	19.80	14.70	2.81	66.50	75.00	59.47	19.68	44.73	48.29	40.83	7.39
53	17.31	21.80	13.60	6.29	67.32	71.60	62.41	8.40	52.28	58.40	46.38	14.52
54	18.87	22.00	15.40	3.90	67.08	69.77	63.75	3.73	49.50	54.44	45.29	9.50
55	15.23	16.90	12.60	1.84	66.92	68.86	63.48	3.50	52.39	57.20	46.94	7.42
56	16.60	20.10	12.90	5.36	66.83	70.87	62.82	7.14	52.76	56.56	49.62	8.18
57	17.74	21.80	13.40	5.95	68.90	79.17	64.12	26.83	50.58	61.29	46.21	17.82
58	17.42	19.60	15.60	1.96	68.38	71.23	66.30	2.90	48.69	55.43	44.21	9.84
59	16.76	19.00	14.70	2.03	66.51	73.80	62.76	9.85	51.78	58.28	47.43	13.19
60	19.78	22.90	17.70	3.15	65.64	68.34	62.61	3.41	50.35	55.14	45.90	10.73
61	19.76	22.70	17.10	3.41	66.91	69.54	63.14	3.92	47.18	53.06	43.85	7.74
62	24.26	27.10	22.10	2.67	64.69	68.34	61.44	4.26	46.45	51.22	41.70	8.31
63	23.48	27.10	20.90	5.51	65.73	74.08	61.52	15.73	49.20	58.91	44.51	18.83

Table 7d. Mean, range and variance for Plant dry weight in mutant families of M₃ generation of Bambara groundnut

	Plant dry weight (g)														
Fam.	Mean	Max.	Min.	Variance	Fam.	Mean	Max.	Min.	Variance	Fam.	Mean	Max.	Min.	Variance	
1	16.87	18.50	14.30	1.41	22	14.87	18.40	9.80	5.91	43	16.69	19.90	13.40	5.55	
2	18.29	20.10	16.20	1.16	23	15.86	20.10	12.60	6.89	44	16.52	19.40	10.60	10.12	
3	17.37	20.50	12.60	4.70	24	15.09	19.60	11.20	6.88	45	18.34	26.20	15.80	10.02	
4	16.20	19.80	12.40	5.91	25	16.58	22.40	11.60	12.29	46	16.59	20.10	12.50	6.74	
5	14.79	19.80	10.90	7.42	26	14.77	18.90	10.30	6.59	47	18.95	24.60	14.20	14.35	
6	15.59	19.80	10.20	9.47	27	15.90	22.30	12.20	6.40	48	18.04	21.30	14.60	5.74	
7	17.24	21.50	11.50	6.83	28	11.57	16.50	7.20	9.32	49	17.84	20.40	14.60	4.72	
8	15.13	18.60	10.50	7.33	29	12.39	18.60	9.60	7.11	50	15.38	17.20	12.30	2.77	
9	16.19	21.20	10.90	10.41	30	12.40	16.10	8.60	7.52	51	19.18	25.40	15.10	9.72	
10	18.11	22.10	13.50	9.01	31	8.34	10.30	6.10	2.52	52	18.74	23.20	12.30	10.34	
11	17.31	22.50	9.50	13.54	32	16.38	20.60	10.30	9.52	53	17.08	20.10	13.50	5.24	
12	16.75	23.40	11.90	14.28	33	17.36	20.10	13.50	6.57	54	18.60	20.10	16.20	2.36	
13	17.83	21.40	13.50	6.74	34	16.61	19.20	13.50	3.14	55	20.70	23.10	16.50	3.76	
14	18.65	23.60	14.20	9.52	35	17.97	22.10	8.90	13.44	56	21.93	26.50	18.90	7.22	
15	16.52	20.10	12.30	6.55	36	15.64	18.60	12.30	2.77	57	18.68	23.10	13.90	6.63	
16	16.81	21.50	13.20	9.26	37	20.61	23.10	18.90	2.45	58	19.72	23.50	15.60	5.31	
17	14.98	20.30	9.80	9.88	38	15.91	20.10	13.20	4.24	59	18.78	22.10	16.50	3.78	
18	17.14	23.50	12.50	12.46	39	16.26	18.90	14.60	1.63	60	21.39	23.60	18.60	3.23	
19	18.37	22.30	12.60	12.28	40	18.30	21.10	15.40	4.27	61	21.84	23.60	19.90	1.96	
20	13.38	16.80	8.90	5.28	41	17.85	21.30	12.50	7.25	62	20.70	23.50	18.60	2.13	
21	17.66	20.40	12.30	6.52	42	20.21	26.50	14.20	12.85	63	21.05	25.60	14.60	12.64	

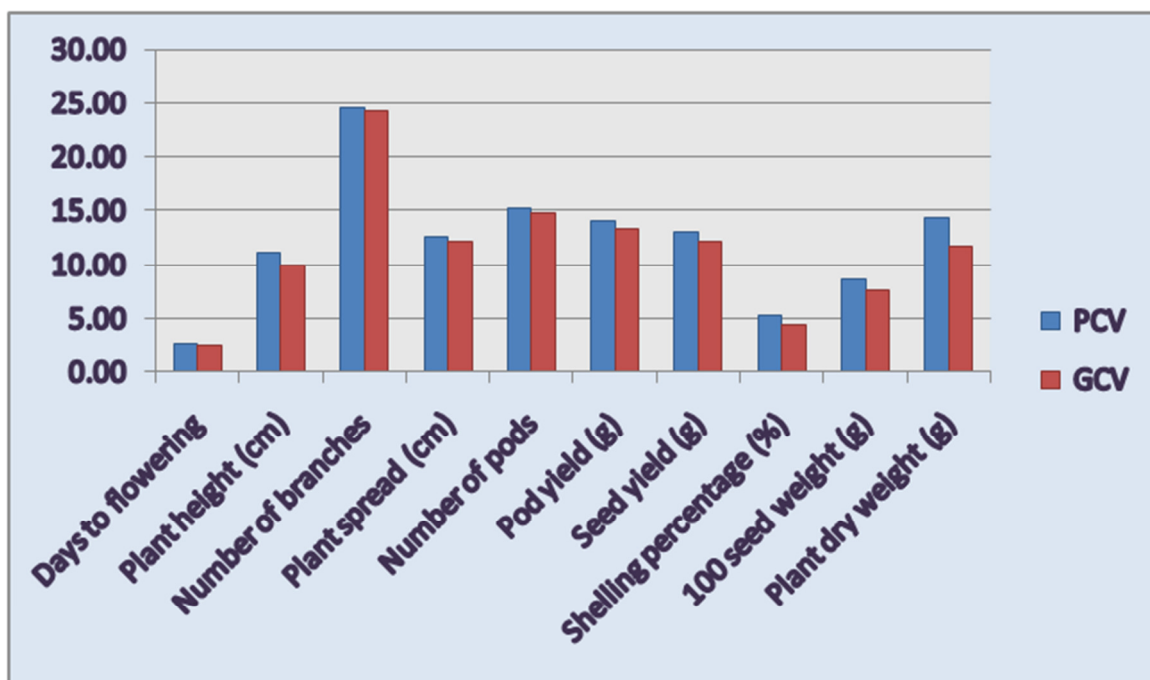


Fig. 2: Estimates of Genotypic coefficient of variance (GCV) and Phenotypic coefficient of variance (PCV)

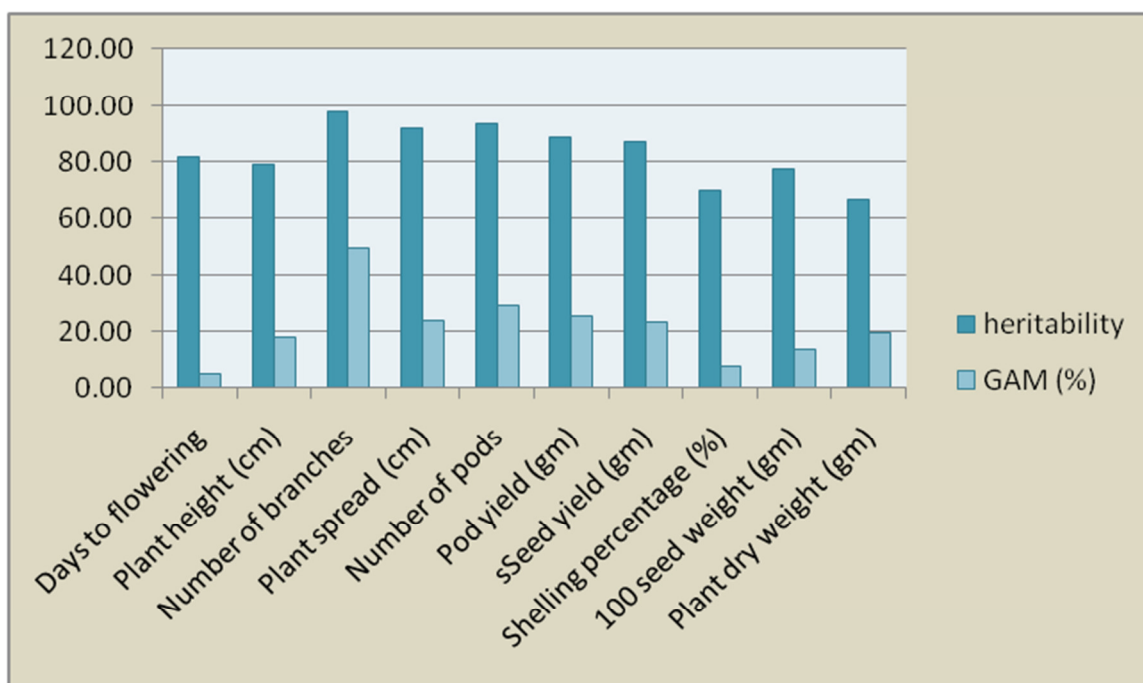


Fig. 3: Estimates of heritability (h^2) and Genetic advance as *per cent* of mean (GAM %)

4.2.3 Number of branches per plant

For this trait a wide range of variation was observed ranging from 25.10 (family 49) to 61.40 (family 59) in the M₃ population with overall mean of 37.31 branches per plant. Wide range was observed in the family 18 (21-35) with high variance of 16.06 among the 63 families.

The genotypic and phenotypic coefficients of variation were 24.22 and 24.53 per cent respectively. This trait had very high heritability (97.52 %) with high genetic advance as *per cent* of mean (49.28).

4.2.4 Plant spread (cm)

Wide range of variation was observed from 15.95 cm (family 28) to 35.51 cm (family 63) for this character with an overall mean of 26.27 cm. Wide range for this trait was observed in family 40 (39-24 cm) and family 44 (36-21 cm) with the variance of 15.69 and 16.54 respectively.

The genotypic and phenotypic coefficients of variation were 12.10 and 12.65 *per cent* respectively. The heritability found to be very high (91.61 %) with high genetic advance as *per cent* of mean (23.86).

4.2.5 Number of pods per plant

Overall mean for this trait was found to be 36.86 with a wide range of variation from 18.90 (family 28) to 52.30 (family 62) pods per plant. Large range was observed in family 53 (25- 47) with a large variation of 40.49.

The genotypic and phenotypic coefficients of variation were 14.75 and 15.28 *per cent* respectively. This trait had very high heritability (93.25 %) with high genetic advance as *per cent* of mean (29.35).

4.2.6 Pod yield per plant (g)

Wide amount of variation varying from 11.96 g (family 28) to 37.51 g (family 62) per plant was observed for this trait with an overall mean of 26.14 g. High range was exhibited by the family 53 (20.30-34.40 g) with a high variance of 20.05 among all the families.

The genotypic and phenotypic coefficients of variation were 13.27 and 14.09 per cent respectively. This trait showed high heritability (88.74 %) coupled with high *per cent* mean genetic advance (14.38).

4.2.7 Seed yield per plant (g)

Seed yield per plant exhibited a wide amount of variation varying from 9.12 g (family 28) to 24.26 g (family 62) per plant with an overall mean of 17.06 g. The family 21 showed wide range of 15.70-25 g with a highest variance of 9.20 in the M₃ population.

The genotypic and phenotypic coefficients of variation were 12.11 and 12.96 per cent respectively. The high heritability (87.28 %) was observed with a high genetic advance as per cent of mean (23.31).

4.2.8 Shelling percentage (%)

An overall mean for shelling percentage was found to be 65.67% with a wide range from 56.64 % (family 19) to 76.87 % (family 28). The family 52 showed maximum range of 59.47-75.00% and the family 57 recorded high variance (26.83) among all the families in M₃ population.

The genotypic and phenotypic coefficients of variation were 4.44 and 5.32 *per cent* respectively. This trait showed high heritability (69.59 %) coupled with low genetic advance as *per cent* of mean (7.62).

4.2.9 Hundred seed weight (g)

An overall mean for 100 seed weight was 46.26 g with a range of 38.23 g (family 21) to 56.55 g (family 3). Wide range for this trait was found to be in the family 5 (36.41-52.86 g) and highest variance was observed in the family 2 (26.23) among all the families.

The genotypic and phenotypic coefficients of variation were 7.65 and 8.70 per cent respectively. The heritability was found to be very high (77.42 %) coupled with moderate genetic advance as per cent of mean (13.87).

4.2.10 Plant dry weight (g)

Wide range of variation was recorded from 8.34 g (family 56) to 21.93 g (family 31) with an overall mean of 17.12 g. Large range was recorded in the family 35 (8.90-22.10 g) and highest variation found in family 11 (13.54 g).

Genotypic and Phenotypic coefficients of variability observed were 11.71 and 14.38 per cent respectively. Heritability of 66.31% coupled with moderate genetic advance per cent of mean (19.64).

4.3 Skewness and Kurtosis

The frequency distribution of M₃population families were positively skewed for the traits like number of branches per plant (0.902), seed yield per plant (0.192), shelling

percentage (0.494) and 100-seed weight (0.129) where as negatively skewed for plant spread(-0.102), number of pods per plant (0.106), pod yield per plant (-0.307) and plant dry weight (-0.69). Traits like plant height (-0.033) and days to flowering (0.003) were near to normal (table 8 & fig. 4a, 4b and 4c).

The frequency distribution of M_3 generation families showed platykurtic distribution for days to flowering (-0.138), plant height (0.656), number of branches per plant (-0.013), plant spread (1.138), number of pods per plant (1.253), shelling percentage (1.693), 100 seed weight (-0.181) and plant dry weight (1.949) where as leptokurtic distribution showed for pod yield per plant (3.769) and seed yield per plant (3.782) (Table 8 & fig. 4a, 4b and 4c).

Table 8: Estimates of quantitative trait Skewness and Kurtosis of the M_3 population derived from SB- 42 variety of Bambara groundnut

Sl. No	Characters	Skewness	Kurtosis
1	Days to flowering	0.00	-0.14
2	Plant height (cm)	-0.03	0.66
3	Number of branches	0.90	-0.01
4	Plant spread (cm)	-0.10	1.14
5	Number of pods	-0.11	1.25
6	Pod yield (gm)	-0.31	3.77
7	Seed yield (gm)	0.19	3.78
8	Shelling percentage (%)	0.49	1.69
9	100 seed weight (gm)	0.13	-0.18
10	Plant dry weight (gm)	-0.69	1.95

Std. Error of Skewness 0.30

Std. Error of Kurtosis 0.59

4.4 Correlation coefficients

In the present investigation, to understand the nature of association existing among the traits, phenotypic correlation was worked among the 10 quantitative traits in M3 generation of Bambara groundnut. The results are presented in the Table 9.

4.4.1 Correlation between seed yield with other related characters

Among 10 quantitative characters, the seed yield showed significantly high positive correlation with pod yield per plant (0.935**) followed by number of pods per plant (0.824**), plant spread (0.675**), plant height (0.681**), plant dry weight (0.476**), and number of branches per plant (0.314*). The characters like days to flowering (0.052), shelling percentage (-0.175) and 100 seed weight (0.031) showed non-significant correlation with seed yield at the phenotypic level.

4.4.2 Correlation among the yield component characters

4.4.2.1 Days to flowering

Days to flowering showed non-significant positive correlations with plant height, number of branches per plant, plant spread, number of pods per plant, pod yield per plant, and plant dry weight and showed negative non-significant correlation with shelling percentage and hundred seed weight.

4.4.2.2 Plant height (cm)

Plant height showed significant and positive correlations with plant spread (0.809**), plant dry weight (0.679**), number of pods per plant (0.648**), pod yield per plant (0.536**) and number of branches per plant (0.312*).

4.4.2.3 Number of branches per plant

Number of branches per plant showed significant and positive correlation with pod yield per plant (0.332**) and plant spread (0.257*).

4.4.2.4 Plant spread (cm)

Plant spread showed significant and positive correlations with pod yield per plant (0.639**), number of pods per plant (0.539**) and plant dry weight (0.552**).

4.4.2.5 Number of pods per plant

Number of pods per plant showed significant positive correlations with pod yield per plant (0.884**), plant dry weight (0.367**) and significant negative correlation with shelling percentage (-0.444**) and 100 seed weight (-0.517**).

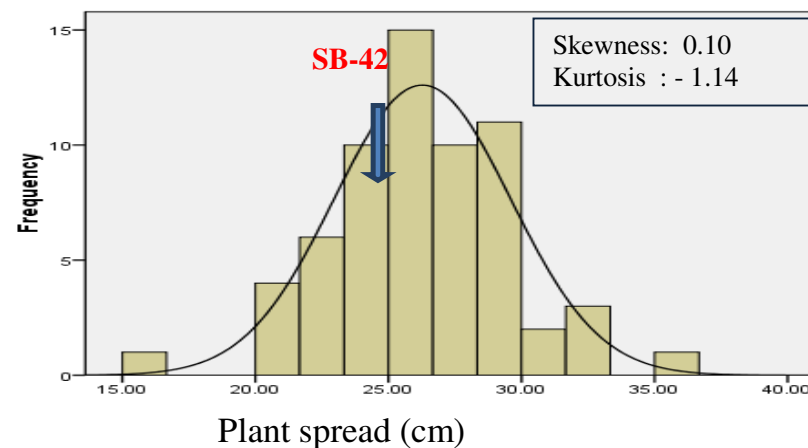
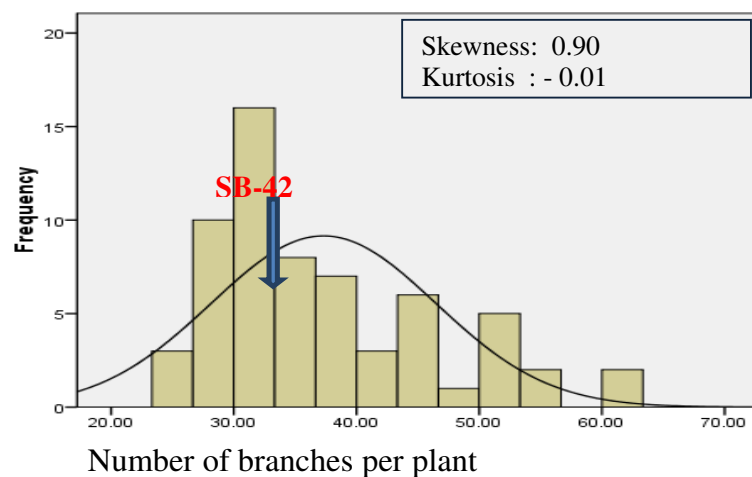
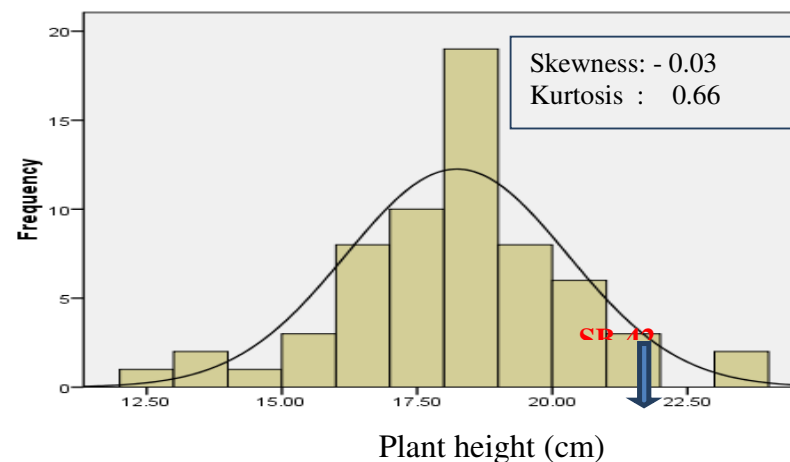
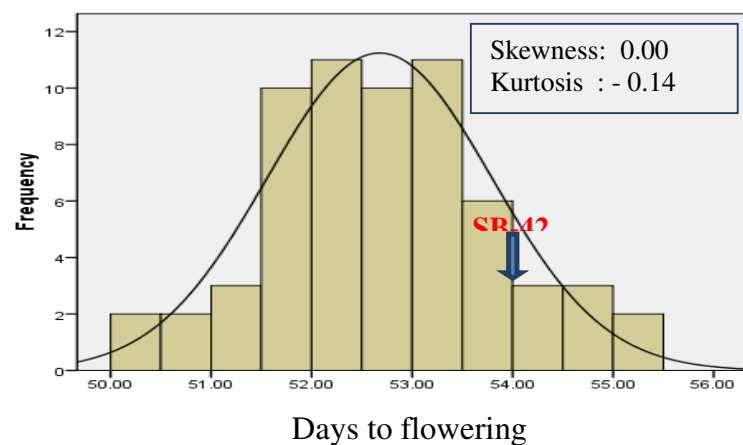


Fig. 4a: Frequency distribution for days to flowering, plant height, branches per plant and plant spread M₃ generation of Bambara groundnut

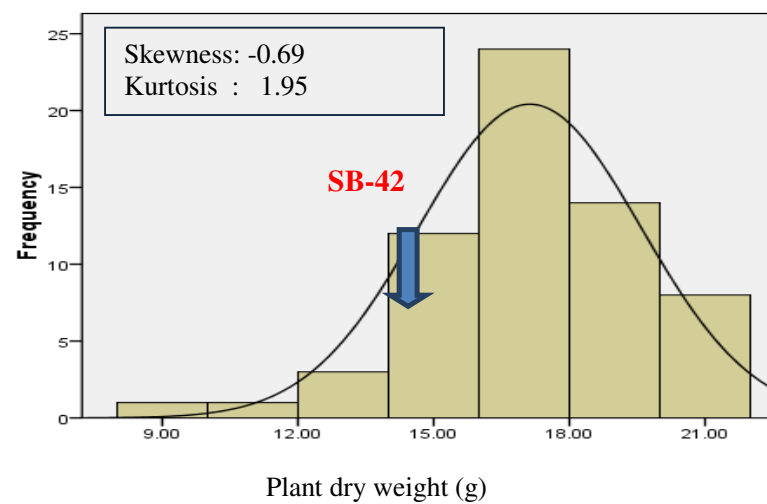
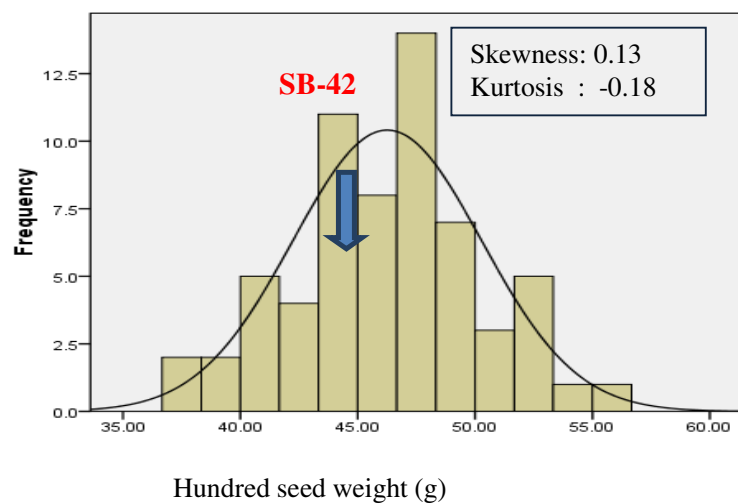
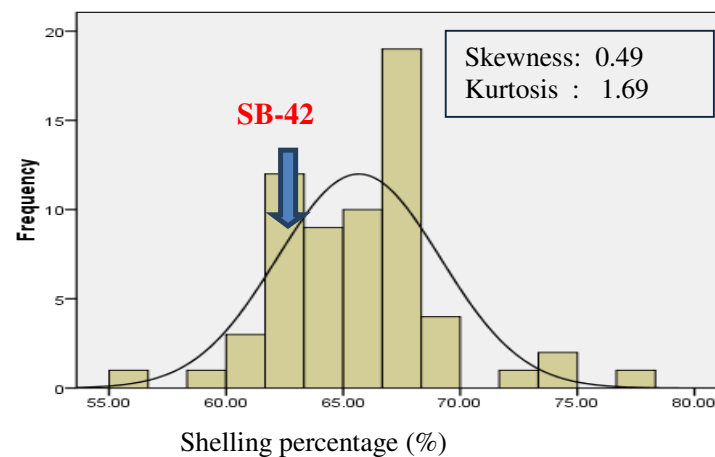
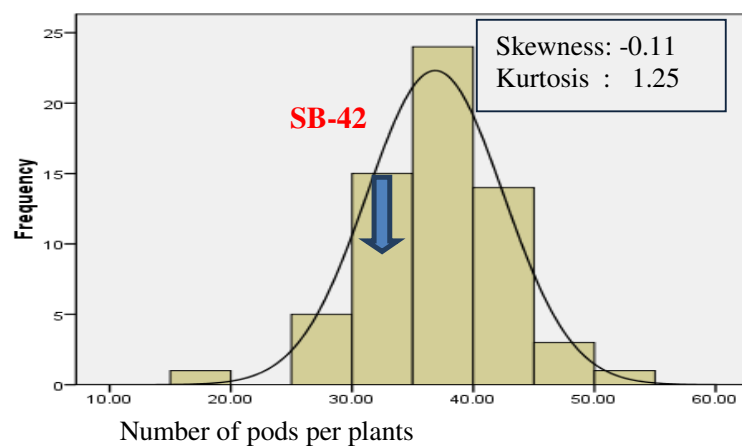


Fig. 4b: Frequency distribution for Number of pods per plant, shelling percentage, hundred seed weight and plant dry weight M_3 generation of Bambara groundnut

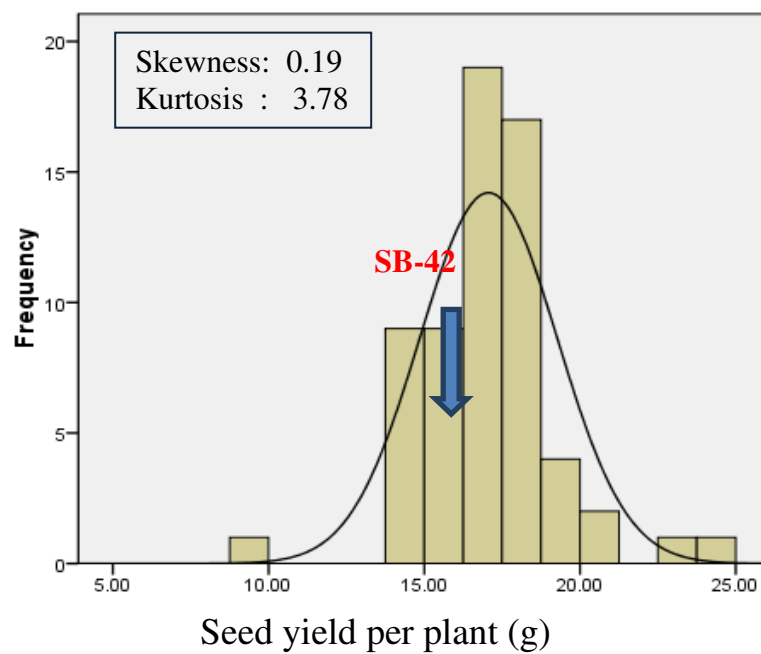
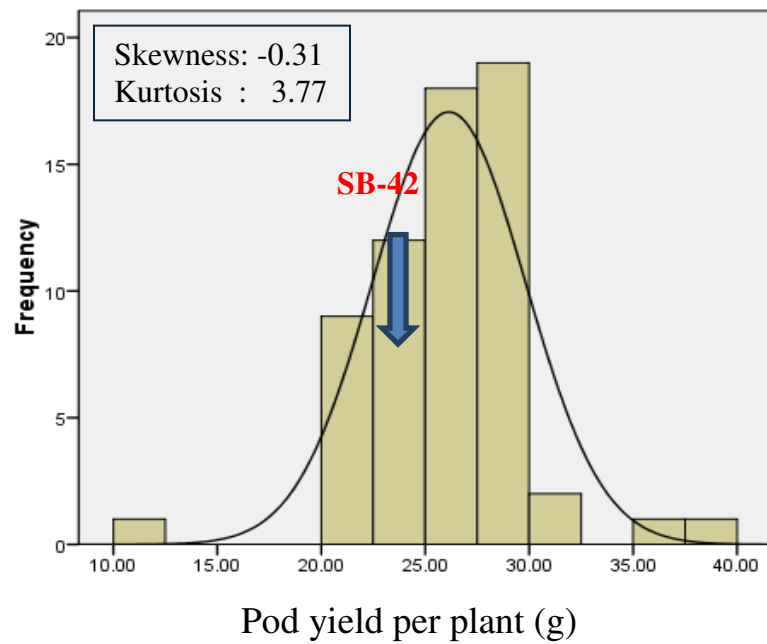


Fig. 4c: Frequency distribution for pod yield and seed yield per plant in M_3 generation of Bambara groundnut

Table 9: Phenotypic correlation coefficient for 10 quantitative characters studied in M₃ generation of Bambara groundnut.

Characters	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀
X ₁	1									
X ₂	0.099	1								
X ₃	0.085	0.312*	1							
X ₄	0.019	0.809**	0.257*	1						
X ₅	0.097	0.536**	0.242	0.539**	1					
X ₆	0.073	0.648**	0.332**	0.639**	0.884**	1				
X ₇	0.052	0.681**	0.314*	0.675**	0.824**	0.935**	1			
X ₈	-0.068	-0.158	-0.151	-0.161	-0.444**	-0.505**	-0.175	1		
X ₉	-0.065	0.082	0.018	0.037	-0.517**	-0.167	0.031	0.530**	1	
X ₁₀	0.122	0.679**	0.183	0.552**	0.367**	0.409**	0.476**	-0.011	0.106	1

**Significant at 0.01

*Significant at 0.05

X₁= Days to flowering

X₂= Plant height (cm)

X₃= Number of branches

X₄= Plant spread (cm)

X₅= Number of pods per plant

X₆=Pod yield per plant (g)

X₇=Seed yield per plant (g)

X₈=Shelling percentage (%)

X₉=100 seed weight (g)

X₁₀=Plant dry weight (g)

4.4.2.6 Pod yield per plant (g)

Pod yield per plant showed highly significant positive correlations with plant dry weight (0.409**) and highly significant negative correlated with shelling percentage (-0.505**).

4.4.2.7 Shelling percentage (%)

Shelling percentage showed highly positive significant correlation with 100 seed weight (0.530**).

4.4.2.8 100 seed weight (g)

Hundred seed weight do not showed any significant correlation with other characters but non-significant positive correlation with plant dry weight (0.106).

4.5 Path coefficient analysis

Path co-efficient analysis was performed at phenotypic levels by considering the seed yield as a dependent trait and remaining nine traits as a independent traits. The results of this analysis are presented in the Table 10 and the direct and indirect effects of different traits on seed yield are presented as follows.

4.5.1 Direct effect of yield contributing characters on seed yield

Six out of 9 characters had positive and direct effect on seed yield at phenotypic level. The characters which had high magnitude positive direct effects are pod yield per plant (1.0596), shelling percentage (0.3644), number of pods per plant (0.0626), 100 seed weight (0.0461), plant spread (0.0243) and plant dry weight (0.0138). While the characters like, plant height (-0.0123), days to flowering (-0.0038) and number of branches per plant (-0.003) had negative direct effects on grain yield. Pod yield per plant (1.0596) had the highest positive direct effect whereas plant height (-0.0123) had the highest negative effect.

4.5.2 Indirect effect of yield contributing characters on seed yield

4.5.2.1 Days to flowering

The indirect and positive effect on seed yield was exhibited by days to flowering through plant spread (0.0005), number of pods per plant (0.0061), pod yield per plant (0.0775) and plant dry weight (0.0017). However, it showed that indirect negative effect on seed yield through plant height (-0.0012), number of branches per plant (-0.0003), shelling percentage (-0.0249) and 100 seed weight (-0.003).

4.5.2.2 Plant height (cm)

The indirect and positive effect on seed yield was exhibited by plant height through plant spread (0.0197), number of pods per plant (0.0336), pod yield per plant

(0.6863), 100 seed weight (0.0038) and plant dry weight (0.0094). However, it showed that indirect negative effect on seed yield through days to flowering (-0.0004), number of branches per plant (-0.0009) and shelling percentage (-0.0577).

4.5.2.3 Number of branches per plant

The indirect and positive effect on seed yield was exhibited by number of branches per plant through plant spread (0.0062), number of pods per plant (0.0152), pod yield per plant (0.3514), 100 seed weight (0.0008) and plant dry weight (0.0025). However, it showed that indirect negative effect on seed yield through days to flowering (-0.0003), plant height (-0.0038) and shelling percentage (-0.0549).

4.5.2.4 Plant spread (cm)

The indirect and positive effect on seed yield was exhibited by plant spread through number of pods per plant (0.0338), pod yield per plant (0.6769), 100 seed weight (0.0017) and plant dry weight (0.0076). However, it showed that indirect negative effect on seed yield through days to flowering (-0.0001), plant height (-0.0099), number of branches per plant (-0.0008) and shelling percentage (-0.0587).

4.5.2.5 Number of pods per plant

The indirect and positive effect on seed yield was exhibited by number of pods per plant through plant spread (0.0131), pod yield per plant (0.9366) and plant dry weight (0.0025). However, it showed that indirect negative effect on seed yield through days to flowering (-0.0004), plant height (-0.0066), number of branches per plant (-0.0007), shelling percentage (-0.1619) and 100 seed weight (-0.0238).

4.5.2.6 Pod yield per plant (g)

The indirect and positive effect on seed yield was exhibited by pod yield per plant through plant spread (0.0155), number of pods per plant (0.0554) and plant dry weight (0.0056). However, it showed that indirect negative effect on seed yield through days to flowering (-0.0003), plant height (-0.0079), number of branches per plant (0.001) shelling percentage (-0.1839) and 100 seed weight (-0.0077).

4.5.2.7 Shelling percentage (%)

The indirect and positive effect on seed yield was exhibited by shelling percentage through days to flowering (0.0003), plant height (0.0019), number of branches per plant (0.0005), and 100 seed weight (0.0244). However, it showed that indirect negative effect on seed yield through plant spread (-0.0039), number of pods per plant (-0.0278), pod yield per plant (-0.5347) and plant dry weight (-0.0001).

Table 10: Estimates of direct and indirect effects of yield components on seed yield at phenotypic level in M₃ generation of Bambara Groundnut

Characters	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	r
X ₁	-0.0038	-0.0012	-0.0003	0.0005	0.0061	0.0775	-0.0249	-0.003	0.0017	0.0525
X ₂	-0.0004	-0.0123	-0.0009	0.0197	0.0336	0.6863	-0.0577	0.0038	0.0094	0.6814**
X ₃	-0.0003	-0.0038	-0.003	0.0062	0.0152	0.3514	-0.0549	0.0008	0.0025	0.3142**
X ₄	-0.0001	-0.0099	-0.0008	0.0243	0.0338	0.6769	-0.0587	0.0017	0.0076	0.6748**
X ₅	-0.0004	-0.0066	-0.0007	0.0131	0.0626	0.9366	-0.1619	-0.0238	0.0051	0.824**
X ₆	-0.0003	-0.0079	-0.001	0.0155	0.0554	1.0596	-0.1839	-0.0077	0.0056	0.9353**
X ₇	0.0003	0.0019	0.0005	-0.0039	-0.0278	-0.5347	0.3644	0.0244	-0.0001	-0.1752
X ₈	0.0002	-0.001	-0.0001	0.0009	-0.0324	-0.1768	0.193	0.0461	0.0015	0.0314
X ₉	-0.0005	-0.0083	-0.0006	0.0134	0.023	0.4337	-0.0039	0.0049	0.0138	0.4756**

RESIDUAL EFFECT = 0.0794

X₁= Days to flowering

X₂= Plant Height (cm)

X₃= Number of branches

X₄= Plant spread (cm)

X₅= Number of pods per plant

X₆=Pod yield per plant (g)

X₇=Shelling percentage (%)

X₈=100 seed weight (g)

X₉=Plant dry weight (g)

4.5.2.8 Hundred seed weight (g)

The indirect and positive effect on seed yield was exhibited by 100 seed weight through days to flowering (0.0002), plant spread (0.0009), shelling percentage (0.193) plant dry weight (0.0015). However, it showed that indirect negative effect on seed yield through plant height (0.001), number of branches per plant (-0.0001), number of pods per plant (-0.0324) and pod yield per plant (-0.1768).

4.5.2.9 Plant dry weight (g)

The indirect and positive effect on seed yield was exhibited by plant dry weight through plant spread (0.0134), number of pods per plant (0.023), pod yield per plant (0.4337) and 100 seed weight (0.0049). However, it showed that indirect negative effect on seed yield through days to flowering (-0.0005), plant height (-0.0083), number of branches per plant (-0.0006) and shelling percentage (-0.0039).

4.6 Molecular screening for yellow mosaic virus disease

Fifty five gamma irradiated M₆ mutant lines of Bambara groundnut (*Vigna subterranea*) were sown during *kharif* 2014 under natural condition at GKVK campus, UAS Bengaluru. These mutant lines were shown resistance and susceptible for yellow mosaic virus under natural condition as reported by Smita (2012).

DNA extracted using modified method of Dellaporta *et al.* (1983) and agarose gel electrophoresis results showing band intensities of DNA isolated from the 55 mutant lines (plate 3) were stable, good quality, purity and suitable for molecular studies. Mutants were screened for YMV disease under the laboratory using five simple sequence repeat (SSR) markers (table 4). None of the designed primers showed amplification for DNA marker linked to the yellow mosaic virus disease resistance.

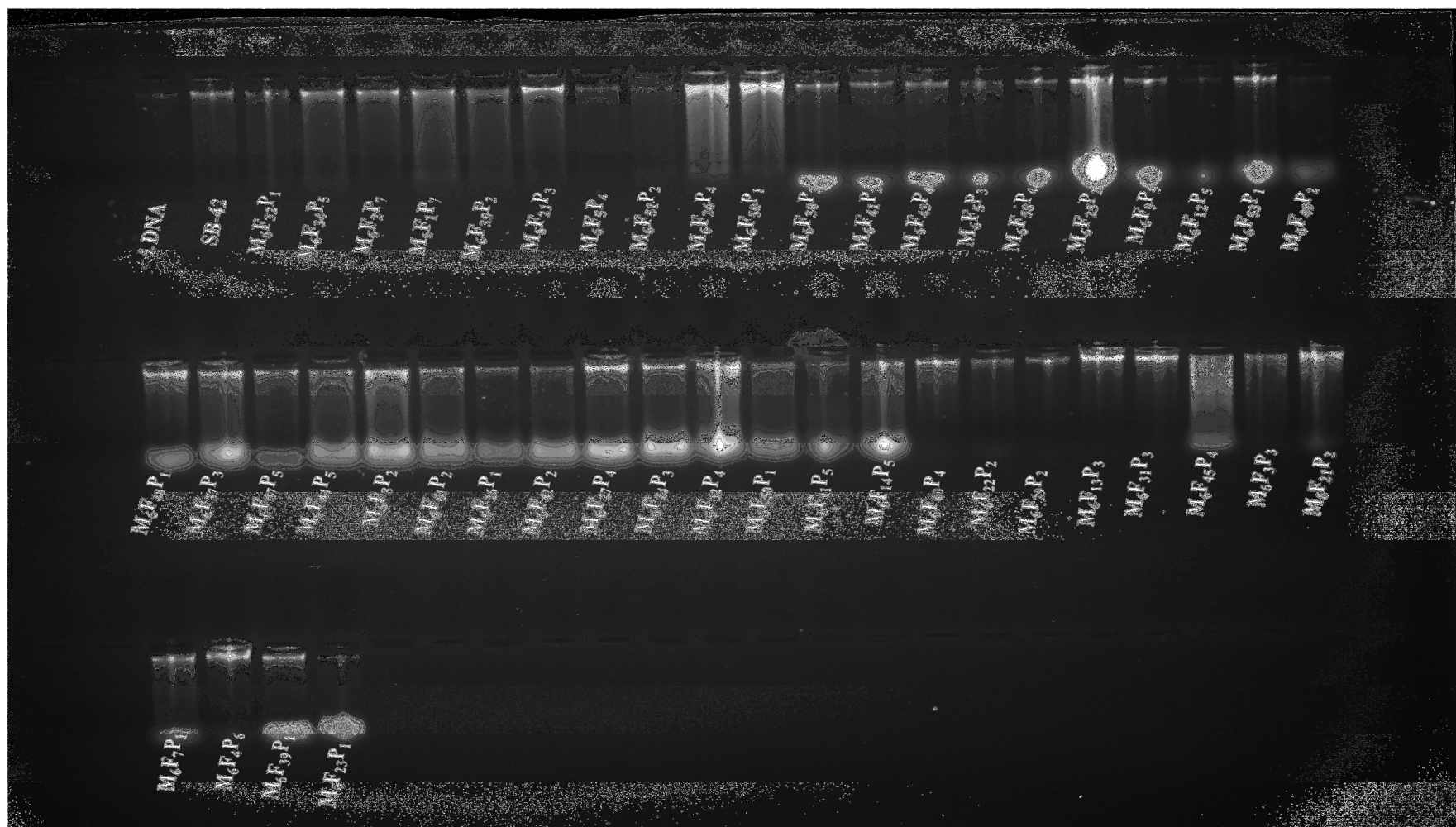


Plate 3: DNA Quantification of M₆ mutant lines.

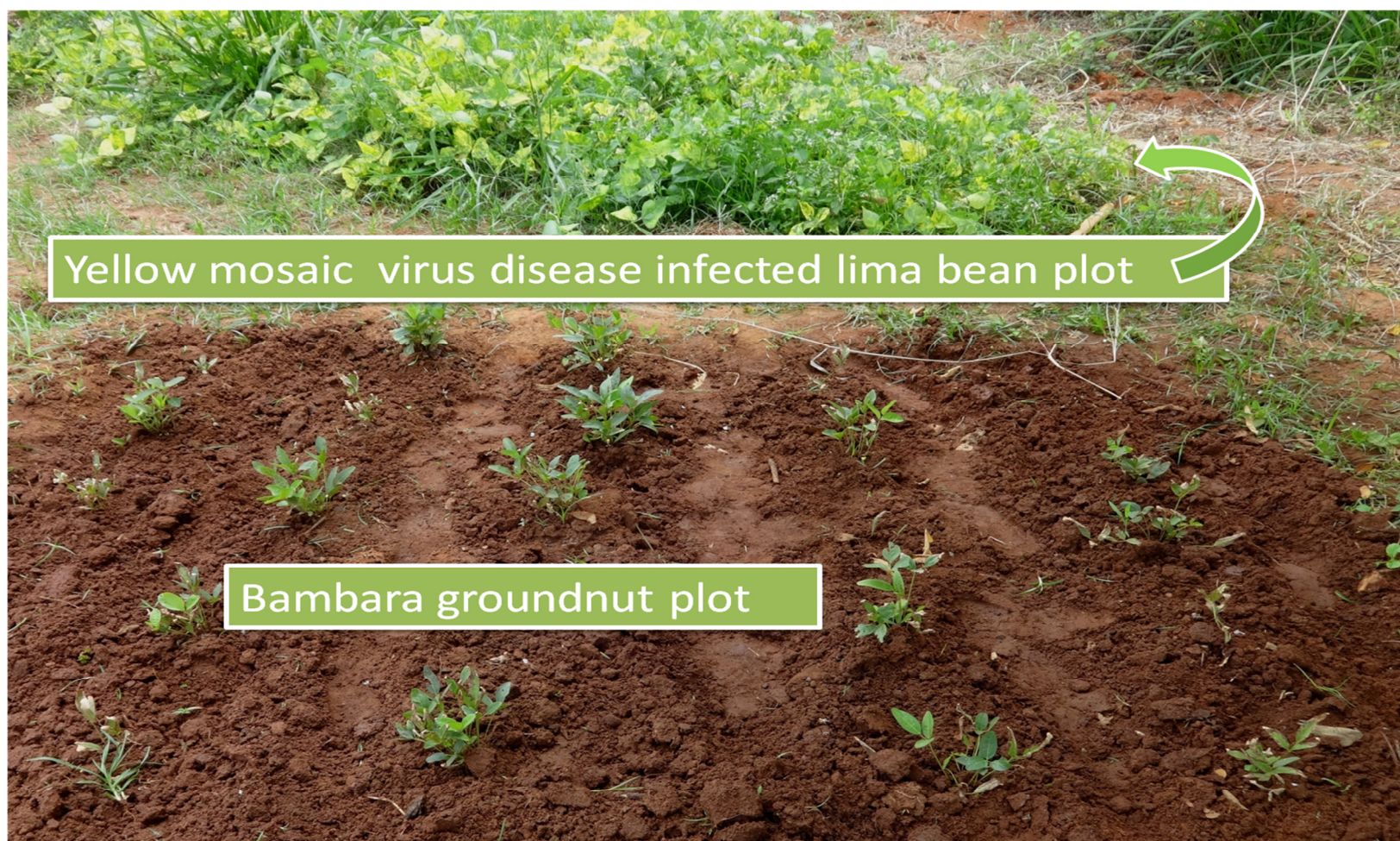


Plate 4: Families of M_6 generation plants showing resistance at field level

V DISCUSSION

For any successful crop improvement programme genetic variability is an essential requirement. The available genetic variability in the plant population today is the result of exposure to natural selection, recombination and spontaneous mutation over centuries. Since Bambara groundnut is an autogamous crop creation of genetic variability is difficult task through conventional breeding methods. Suwanprasert *et al.* (2006) reported as it is difficult to undertake hybridization. Hence, induced mutation has been used as a practical tool for creation of genetic variability to isolate the desirable mutant lines *i.e.*, early maturity, higher yield, disease resistance and many more characters. This creates variation within the crop variety and it offers the possibility of inducing desired changes in attributes to select improved cultivars and also for generating novel mutant traits.

The knowledge of genetic variability in population with respect yield and its components, their magnitude of association, nature and extent of relative contribution towards seed yield and amount of deviation of these characters over a environment are very much essential for planning successful breeding programme.

Breeding for biotic and abiotic resistance is also major objective of the breeders to develop resistant varieties using advanced studies like marker assisted selection to overcome the inaccuracy of field experiment. In accordance with these considerations we conducted screening for yellow mosaic virus disease resistance because it is a major threat to pulse production in Indian agriculture. Considering these views, sixty three M_3 families of Bambara groundnut were evaluated for yield and its components and M_6 mutants were utilized to conduct studies on yellow mosaic virus disease resistance using SSR markers.

The results obtained from the present investigation are discussed under the following headings.

5.1 Comparison of mean performance of mutants with the parent

5.2 Genetic variability parameters in M_3 generation

5.3 Skewness and Kurtosis

5.4 Correlation coefficients

5.5 Path coefficient analysis

5.6 Molecular screening for yellow mosaic virus disease

5.1 Comparison of mean performance of mutants with the parent

Mutant population displayed significant difference between the mean values for all the quantitative characters studied except hundred seed weight.

Mean performance of the characters like number of branches per plant (37.31), plant spread (26.27 cm), number of pods per plant (36.86), pod yield per plant (26.14 g), seed yield per plant (17.06 g), shelling percentage (65.67 %) and plant dry weight (17.12 g) were significantly increased compared to the parent. An increase in variation for these characters in the population indicates a good scope for selection of superior and suitable mutant lines as per our requirement. Similar results were also reported by Mohd rafiq and samiullah (2006) in mung bean, Singh *et al.* (2013) in black gram for number of branches per plant; Adudapaah and Sangwan (2004) in Bambara groundnut and Mahammad Yaqoob (2003) in mung bean reported increased mean values for plant spread; Vijaykumar (2011) in Bambara groundnut, Mohdrafiq and samiullah (2006) in mung bean, Henry (2012) and Singh *et al.* (2013) in cowpea reported for increased mean values for number of pods per plant and increased variability; Arulbalchandran and mullainathan (2009) in black gram reported increased mean value for pod yield per plant; Adudapaah and Sangwan (2004) and Spreeth and De ronde (2004) in Bambara groundnut reported increased mean values and increased variability for seed yield per plant; Vijaykumar (2011) and Smita (2012) in Bambara groundnut reported increased mean value for shelling percentage and 100 seed weight.

Days to flowering (53.03 days) and plant height (18.24 cm) showed negative shift in mean *viz.*, decreased mean value compared to parent. M_3 population showed one day early flowering compared to the parent is more desirable in plant breeding programme which can be exploited by selection process for further generation. Similar results are confirmed with the findings of Adu-dapaah *et al* (2003), Smita (2012) in Bambara groundnut and Sri Devi and Mullainathanan (2012) in Black gram and contradictory results in Vijaykumar (2011) studies in Bambara groundnut.

5.2 Genetic variability parameters in M_3 generation

Improvement of any crop depends on the existence of genetic variability in population and extent to which traits are heritable. Greater the genetic variability present in the population greater the efficiency of selection programme. It is very essential to understand the influence of the environmental variance in the present variation to precise our selection programme. The estimate of heritability alone is not sufficient to predict transfer of traits to next generation. Hence, heritability estimates appears to be more meaningful when accompanied by the estimate of genetic advance (Johnson *et al.*, 1955). All the mutant families in M_3 showed a wide range of variation for all characters which offers scope for selection of superior and desired mutants by the plant breeders for further improvement of the crop. Higher genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) were reported with reference to the number of

branches per plant (24.22 & 24.53). While a moderate GCV and PCV were recorded for plant height (9.92 & 11.16), plant spread (12.10 & 12.65), number of pods per plant (14.75 & 15.28), pod yield per plant (13.27 & 14.09), seed yield per plant (12.11 & 12.96) and plant dry weight (11.71 & 14.38) indicates the considerable variability in the population which will be helpful in selection programme. A low GCV and PCV were recorded for days to flowering (2.42 & 2.67), shelling percentage (4.44 & 5.32) and hundred seed weight (7.65 & 8.70) these results are contradictory to the findings of Smita (2012). Genotypic coefficient of variability (GCV) would be more useful for the assessment of heritable or true variability. The differences between the estimated GCV and PCV for number of branches per plant were found to be very less where as for plant dry weight found to be high indicates genes responsible for number of branches per plant was less influenced by the environment and genes responsible plant dry weight affected by most. It is assumed that the phenotypic variability can be utilized directly in selection of mutant lines.

Broad sense heritability was estimated for all the characters studied and it was found to be high for all the characters. Hence, such heritability suggests greater effectiveness of selection for superior and suitable mutant lines and improvement to be expected for these characters in future breeding programme as the genetic variance is mostly is due to additive gene expression. In the present investigation, high heritability coupled with high genetic advance as *per cent* over mean was observed for the number of branches per plant (97.52 & 49.28), plant spread (91.61 & 23.86), number of pods per plant (93.25 & 29.35), pod yield per plant (88.74 & 25.75), seed yield per plant (87.28 & 23.31). The results are in accordance with the results obtained by Smita (2012) who observed high heritability with GAM for number of branches per plant, plant spread, pods per plant, pod yield per plant, seed yield per plant. This reveals that lesser role of environment on expression of these traits and prevalence of additive gene action in their inheritance, hence they are more reliable for selection. A high genetic advance over mean coupled with high heritability suggested the importance of additive gene action for these traits. The high heritability with moderate genetic advance for plant height, 100 seed weight and plant dry weight suggesting that some amount of heritability due to environment. Similar results were reported from Imran *et al.* (2014) in mung bean. A high heritability with low genetic advance as *per cent* of mean for the days to flowering and shelling percentage characters in the population indicating the presence of non additive gene action and role of environment in expression of these traits. These results are accordance with the findings of Imran *et al.* (2014) for these two traits. Hence, preference for selection should be given to those traits which recorded higher estimates heritability coupled with high genetic advance as *per cent* mean and selection based on these characters may be useful in realizing better gain by selection.

5.3 Skewness and Kurtosis

In M₃ population positive skewness was observed for number of branches per plant, shelling percentage, 100-seed weight and seed yield per plant indicating the

existence of complementary epistasis for expression of these characters, where as negative skewness observed for plant spread, number of pods per plant, pod yield per plant and plant dry weight suggesting that these traits are displaying duplicate epistasis. Since these traits are affected it could be probably due to mutation.

Platykurtic distribution was observed for traits like days to flowering, plant height, number of branches per plant, plant spread, number of pods, shelling percentage, 100 seed weight and plant dry weight indicating these characters governed by large number of genes and displaying complementary epistasis, where as pod yield per plant and seed yield per plant showed positively skewed leptokurtic distribution suggesting that traits are governed by few number of genes showing complementary gene action.

5.4 Correlation coefficients

5.4.1 Correlation between yield and yield components

Nature of association of character between yield and its components is highly essential for selection for more than one character. The correlation coefficient analysis is a basic concept to know the direction and number of characters to be considered for improvement in yield.

In the present investigation, correlations among 10 quantitative characters of Bambara groundnut (*Vigna subterranea*) were analysed. The seed yield had significantly high positive correlation with the pod yield per plant (0.935) followed by number of pods(0.824), plant height(0.681), plant spread(0.675), plant dry weight(0.476) and number of branches per plant(0.314). Such highly significant positive correlation with pod yield per plant has also been reported by Makanda *et al.* (2009), Vijaykumar (2011) and Jonah (2012) in Bambara groundnut. Thus these reports suggest that lesser thickness of shell is one of the indirect measures to select higher seed yield. Therefore it would be desirable for selection of plant. The significantly high positive correlation between the number of pods per plant and seed yield per plant suggesting that as the number pods increase seed yield will also increases. Similar findings also reported by Karikari (1972), Ofori (1996), Goli *et al.* (1997), Makanda *et al.* (2009), Benedict *et al.* (2011) and Maunde *et al.* (2015) in Bambara groundnut.

Plant height plant spread also had significant correlation with the seed yield which indicates that these character to be selected for high yield. Thus it would be desirable to select plants having good height and maximum plant spread. Such results have also been documented by Parameswarappa and kumar (2005) in black gram and Diriba *et al.* (2014) in cowpea.

Number of branches per plant was found to behave positively and significantly with seed yield indicates the seed yield increases with increase in number of branches as reported by Punia *et al.* (2014) in black gram and Diribi *et al.* (2014) in cowpea.

Plant dry weight was found to be have positively and significantly correlated with seed yield indicates that increase in biomass leads to the increase in seed yield, such reports have also been documented Narasimhulu *et al.* (2013) in green gram and Chauhan *et al.* (2007) in black gram.

5.4.2 Correlation among yield components

Understanding of association of yield component traits help to identify an ideal plant type. Plant height showed significant and positive correlations with plant spread, plant dry weight, number of pods per plant, pod yield per plant and number of branches per plant. Number of branches per plant showed significant and positive correlation with pod yield per plant and plant spread indicates the as branches number increases with increase in the pod yield so it is an indirect selection for a seed yield. These results are similar with the findings of Diribo *et al.* (2014) in cowpea. Plant spread showed significant and positive correlations with pod yield per plant, number of pods per plant and plant dry weight. Number of pods per plant showed significant positive correlations with pod yield per plant and plant dry weight. Diribo *et al.* (2014) in cowpea. Number of pods per plant had significant negative correlation with shelling percentage and 100 seed weight. These results were similar with the findings of Jonah *et al.* (2010). Pod yield per plant showed highly significant positive correlations with plant dry weight. These results were in accordance with the study of Smita (2014). Pod yield had highly significant negative correlated with shelling percentage. Shelling percentage showed highly positive significant correlation with 100 seed weight.

Therefore, from the present study it can be concluded that, greater importance has to be given to the characters *viz.*, pod yield per plant, number of pods, plant height, plant spread, plant dry weight and number of branches per plant during selection process for improving seed yield per plant as these characters show positive and highly significant correlation with seed yield per plant.

5.5 Path coefficient analysis

Correlation coefficient explains the nature and extent of association existing between pairs of characters. Economic characters like seed yield are dependent on its related component characters. Therefore character has to be analyzed for its effect through path analysis. There are two types of effects *viz.*, direct effect of component character on seed yield and the indirect effects through other component characters on seed yield are obtained which cannot be understood by the correlation studies. Path analysis was first suggested by Wright (1921). It is standardized partial regression analysis based on cause and effect of relationship and is useful for analysis by subdividing correlation in a causal scheme.

In the present study out of nine quantitative characters six showed the significant association with the seed yield. These characters were subjected to path analysis to

determine direct and indirect effect and the results are discussed here. In M₃ population path analysis revealed that, the characters like pod yield per plant (1.0596), shelling percentage (0.3644), number of pods per plant (0.0626), 100 seed weight (0.0461), plant spread (0.0243) and plant dry weight (0.0138) had positive direct effect on seed yield while, plant height (-0.0123), days to flowering (-0.0038) and number of branches per plant (-0.003) had negative direct effects on seed yield. Among these pod yield per plant had maximum positive effect and plant height had maximum negative effect on seed yield. Highly significant correlation between pod yield per plant and seed yield was observed. This positive correlation was due to the direct effect of pod yield per plant on seed yield. Indirect positive effect via plant spread, number of pods per plant and plant dry weight while Indirect negative effect via days to flowering, plant height, number of branches per plant, shelling percentage and 100 seed weight. Highly significant correlation between number of pods per plant and seed yield was observed. This positive correlation was mainly due to the indirect positive effect of pod yield per plant on seed yield. Indirect positive effect via pod yield per plant, plant spread and plant dry weight while Indirect negative effect via days to flowering, plant height, number of branches per plant, shelling percentage and 100 seed weight. Highly significant correlation between plant spread and seed yield was observed. This positive correlation was mainly due to the indirect positive effect of pod yield per plant on seed yield. Indirect positive effect via pod yield per plant, number of pods per plant, 100 seed weight and plant dry weight while Indirect negative effect via days to flowering, plant height, number of branches per plant and shelling percentage. Highly significant correlation between number of branches per plant and seed yield was observed. This positive correlation was mainly due to the indirect positive effect of pod yield per plant on seed yield and indirect positive effect via pod yield per plant, plant spread, number of pods per plant, 100 seed weight and plant dry weight while Indirect negative effect via days to flowering, plant height and shelling percentage. Highly significant correlation between plant dry weight and seed yield was observed. Such positive correlation was mainly due to the indirect positive effect of pod yield per plant on seed yield. Indirect positive effect via pod yield per plant, plant spread, number of pods per plant and 100 seed weight while Indirect negative effect via days to flowering, plant height, number of branches per plant and shelling percentage. These results are similar in accordance with result of Vijaykumar (2011) and Smita *et al.* (2014) in gamma irradiated Bambara groundnut.

5.6 Molecular screening for yellow mosaic virus disease

Yellow mosaic virus disease is one of the most devastating disease causing hundred per cent economic loss in most of the *Vigna spp.* Hence there is need of developing resistant varieties. YMV disease was reported by Muniyappa and Veeresh

(1984) in Bambara groundnut from the field of Agriculture farm, University of Agriculture sciences, Bangalore in 1981 and confirmed its transmittance principally by whitefly, (*Bemisia tabaci*). Later Smita (2012) also reported similar findings. Her studies indicated susceptible and resistant mutant lines for the yellow mosaic virus disease in the gamma irradiated mutant population. In this contest we undertook screening of YMV disease in laboratory condition by using the SSR markers designed in NCBI website.

Five SSR primers were designed by using the pick primer tool in the NCBI website for the RGA primer RGA 1F-CG/RGA 1R sequence reported by Basak *et al.* (2004) considered in our study to screen the mutant population treated with gamma radiation. However all the primers were unable to amplify these mutant lines which infers that these markers are either not sufficient to identify the linked marker DNA to yellow mosaic virus disease resistance gene in Bambara groundnut or it is possible that all the genotypes screened were susceptible. Hence, an array of heterologous probes with large number of mutants needs to be screened.

Future line of work

1. The families SB42-M₃-F P_{9 8} (24.26), SB42-M₃-F P_{4 1} (23.48), SB42-M₃-F P_{11 2} (21.04), SB42-M₃-F P_{17 1} (20.08) and SB42-M₃-F P_{8 7} (19.78) are isolated for higher seed yield per plant and will be exploited in future selection programme.
2. Adequate amount of variability in the M₃ mutant population exist which can be considered for further selection programme.
3. The study of correlation coefficient and path analysis for pod yield per plant, number of pods per plant, plant spread and plant dry weight can be used as important selection criterion in future breeding programme
4. In future, large number lines should be screened for yellow mosaic virus disease resistance using a spectrum of markers.

VI SUMMARY

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an important legume crop of African continent majorly grown for its grains by resource poor farmers. It is hardy crop, good yielder under the drought and poor soil condition. The yield potential of this crop is low and paused because of limited genetic variability and number of disease. Since it is an autogamous crop conventional methods of plant breeding have a limited importance for development of new varieties like high yielding and disease resistance varieties.

The present study was under taken to assess genetic variability in sixty three M_3 families, to understand trait association between seed yield and its attributes, to know direct and indirect effect between seed yield and its component characters and to screen for yellow mosaic virus disease resistance in fifty five M_6 mutant lines and to isolate high yielding and yellow mosaic virus disease resistance mutants for future breeding programmes. Results obtained in this study are summarized below.

It was found that mean performance of M_3 mutant population significantly increased for the characters like number of branches per plant, plant spread, number of pods per plant, pod yield per plant, seed yield per plant shelling percentage and plant dry weight while days to flowering and plant height were significantly decreased with increase in variability. This indicates the effectiveness of mutation in creating genetic variability. Higher GCV and PCV were observed for number of branches per plant. While moderate GCV and PCV were observed for plant height, plant spread, number of pods per plant, pod yield per plant, seed yield per plant and plant dry weight. A low GCV and PCV were recorded for days to flowering, shelling percentage and hundred seed weight. The difference between PCV and GCV was found to be narrow indicating lesser role of environment over expression of these characters. Heritability in broad sense was found to be higher for most of the character. Higher heritability coupled with high genetic advance percent of mean was observed for the number of branches per plant, plant spread, number of pods per plant, pod yield per plant, seed yield per plant. While plant height, 100 seed weight and plant dry weight recorded high heritability with moderate genetic advance. A high heritability with low genetic advance as *per cent* of mean was recorded for days to flowering and shelling percentage. Hence most of the characters are considered to be governed by the additive gene action and amenable for selection. In M_3 population positively skewed platykurtic distribution was observed for number of branches per plant, shelling percentage, and 100-seed weight, where as negatively skewed platykurtic distribution for plant spread, number of pods per plant and plant dry weight. Traits like pod yield per plant and seed yield per plant showed positively skewed leptokurtic distribution and normal platykurtic distribution was observed for plant height and days to flowering.

Seed yield per plant had positive significant association with six out of nine characters studied are pod yield per plant, number of pods per plant, plant height, number of branches per plant, plant spread and plant dry weight. These characters provide an opportunity for selection of high yielding genotype.

Path coefficient analysis indicates that pod yield per plant, shelling percentage, number of pods per plant, plant spread, 100 seed weight and plant dry weight had high of direct effect positive on seed yield. Such characters will be useful in selection programme for increasing yield, while other characters *viz.*, plant height, days to flowering and number of braches per plant had negative direct effects on seed yield, in spite of their positive genotypic correlation with grain yield. Thus correlation and path coefficient analysis together will draw conclusive relationship between the characters.

Five SSR primers were designed using the pick primer tool from the NCBI website for the RGA primer RGA 1F-CG/RGA 1R sequence reported by Basak *et al.* (2004) in *Vigna mungo* due to its sequence similarity found in many of the *Vigna* species blasted. Hence the same sequence was used to design primers. These heterologous primers were used to screen the isolated and stabilized mutants of Bambara groundnut. However all the designed primers were unable to amplify the mutant lines which infers that either all the lines lack resistance or these markers are not sufficient to identify the linked marker to yellow mosaic virus disease resistance in Bambara groundnut. Hence an array of heterologus probes with large number of mutants need to screen.

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ANNEXURE I

I. Buffers and solutions used for isolation of DNA.

1) Cetyl Trimethyl Ammonium Bromide (CTAB) extraction buffer

i. CTAB	2%(w/v)
ii. Tris- HCl (Ph 8.0)	200mM
iii. NaCl (Sodium Chloride)	1.4M
iv. EDTA (Ethylenediaminetetra acetic Acid)	20 Mm
v. PVP (polyvinylpyrrolidone)	1% (v/v)
vi. β -mercaptoethanol	0.1% (v/v)

2) Tris- EDTA (TE) Buffer

i. Tris-HCL (pH 8.0)	10 mM
ii. EDTA (Ph 8.0)	1 mM

- 3) Chloroform: Isomylalcohol 24:1 (v/v)
- 4) Phenol:Chloroform 1:1 (v/v)
- 5) Sodium acetate 3.0 M (pH 5.2- adjusted using glacial acetic acid)
- 6) Ethanol 100% and 70%
- 7) RNase 10 mg/ml
- 8) Ice-cold Isopropanol
- 9) Liquid nitrogen