GT BIPLOT ANALYSIS FOR YIELD TRAITS AND MOLECULAR PROFILING OF SELECTED LINES IN BLACKGRAM [*Vigna mungo* (L.) Hepper]

BY

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B.Sc. (Hons.) Ag.

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DECLARATION

I, **Ms. DIGUMARTHI LAKSHMI VINEESHA** hereby declare that the thesis entitled **"GT BIPLOT ANALYSIS FOR YIELD TRAITS AND MOLECULAR PROFILING OF SELECTED LINES IN BLACKGRAM [*Vigna mungo* (L.) Hepper]"** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

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Ms. DIGUMARTHI LAKSHMI VINEESHA has satisfactorily prosecuted the course of research and that thesis entitled **"GT BIPLOT ANALYSIS FOR YIELD TRAITS AND MOLECULAR PROFILING OF SELECTED LINES IN BLACKGRAM [*Vigna mungo* (L.) Hepper]"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

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This is to certify that the thesis entitled **"GT BIPLOT ANALYSIS FOR YIELD TRAITS AND MOLECULAR PROFILING OF SELECTED LINES IN BLACKGRAM [*Vigna mungo* (L.) Hepper]"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N.G. Ranga Agricultural University, Lam, Guntur is a record of bonafide original research work carried out by **DIGUMARTHI LAKSHMI VINEESHA** under our guidance and supervision.

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LIST OF SYMBOLS AND ABBREVIATIONS

$\overline{X}$	:	Grand mean
%	:	Per cent
<	:	Less than
>	:	Greater than
°	:	Degree
°C	:	Degree Celsius
µ	:	Micron
µl	:	Micro Litre
100 SW	:	Hundred Seed Weight
ANOVA	:	Analysis of variance
ARS	:	Agricultural Research Station
ATA	:	Average Tester Axis
ATC	:	Average Tester Coordinate
bp	:	base pair
CD	:	Critical Difference
cm	:	Centimeter
CTAB	:	Cetyl Trimethyl Ammonium Bromide
CV	:	Coefficient of Variation
df	:	Degree of Freedom
DFF	:	Days to 50% flowering
DM	:	Days to Maturity
DNA	:	Deoxyribonucleic Acid
dNTP	:	Deoxy Nucleotide Tri Phosphate
EDTA	:	Ethylene Diamine Tetra Acetic Acid
ESS	:	Error Sum of Squares
et al.	:	and other people
Fig.	:	Figure
g	:	Gram
GA	:	Genetic Advance

GAM	:	Genetic Advance as per cent of Mean
GCV	:	Genotypic Coefficient of Variation
GT	:	Genotype by Trait
ha	:	Hectare
HI	:	Harvest Index
h^2_b	:	Heritability in broad sense
i.e.	:	that is
kg ha^{-1}	:	Kilograms per hectare
L	:	Litre
LG	:	Linkage group
M	:	Molar
mM	:	Milli molar
mg	:	Milligram
min	:	Minutes
ml	:	Millilitre
MSS	:	Mean Sum of Squares
ng	:	Nanogram
nm	:	Nanometer
NCP	:	Number of Cluster per Plant
NPBP	:	Number of Primary Branches per Plant
NPC	:	Number of Pods per Cluster
NPP	:	Number of Pods per Plant
NSP	:	Number of Seeds per Pod
PC	:	Principal Component
PCR	:	Polymerase Chain Reaction
PCV	:	Phenotypic Coefficient of Variation
PIC	:	Polymorphic Information Content
PH	:	Plant Height
pH	:	Potential of hydrogen
PL	:	Pod Length
pmole	:	Picomole

PPV&FR	:	Protection of Plant Varieties and Farmer's Right
per se	:	As such with mean
RARS	:	Regional Agricultural Research Station
RBD	:	Randomized Block Design
RSS	:	Replication Sum of Squares
rpm	:	rotations per minute
r_g	:	Genotypic correlation coefficient
r_p	:	Phenotypic correlation coefficient
S.E (d)	:	Standard Error of difference
S.E (m)	:	Standard Error of mean
S.No	:	Serial Number
SSR	:	Simple Sequence Repeats
SVP	:	Singular Value Partitioning
SYP	:	Seed Yield per Plant
TBE	:	Tris Borate EDTA
TSS	:	Total Sum of Squares
V	:	Volts
v/v	:	Volume by volume
VSS	:	Varietal Sum of Squares
via	:	Through
viz.,	:	Namely
σ	:	Standard Deviation
σ^2	:	Variance

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ABSTRACT

The present investigation was carried out at dryland farm of Sri Venkateswara Agricultural College, Tirupati during *Rabi*, 2021-22 to study genetic parameters, genetic diversity, genotype by trait biplot analysis for twelve traits and to develop molecular profiles of 35 blackgram genotypes. Moderate estimates of GCV and higher estimates of PCV were observed for the traits plant height, number of pods per plant, number of clusters per plant and harvest index. High heritability coupled with high genetic advance as per cent of mean was recorded for number of primary branches per plant. Genetic divergence analysis using Mahalanobis D^2 statistics grouped 35 genotypes into eight clusters. Among the clusters, cluster III had the maximum intra cluster distance followed by cluster I and cluster V. The maximum inter cluster distance was recorded between cluster IV and V followed by cluster V and VIII, cluster I and V, cluster V and VII and cluster VII and VIII. Hence, the crosses *viz.*, LBG 645 × P 112, LBG 645 × IP4-10-4, LBG 645 × PU 31, LBG 645 × P 1032 and P 1032 × IPU-10-4 could be suggested for the exploitation of transgressive segregants for yield.

Based on genotype by trait biplot analysis, the seed yield was positively correlated with number of pods per plant, number of clusters per plant, number of primary branches per plant, number of seeds per pod, plant height, days to maturity, pod length, number of pods per cluster and harvest index. The trait number of primary branches per plant was considered as best, similarly, the genotype LGG 645 was identified as ideal cultivar.

Molecular profiling disclosed, out of 16 SSR markers, ten were polymorphic and generated unique DNA profiles for 35 blackgram genotypes studied. Based on the level of polymorphism detected by individual marker, four SSR markers (CEDG 20, CEDG 44, CEDG 243 and BM 170) were most informative and this set of markers had the enormous potential to identify most of the blackgram genotypes studied.

Chapter - I

Introduction

Chapter – 1

INTRODUCTION

Blackgram (*Vigna mungo* L. Hepper), a diploid (2n = 2X = 22), short duration legume crop of family *Fabaceae*, was domesticated in Northern South Asia from progenitor *Vigna mungo* var. *silvestris* (Lukoki *et al.,* 1980). It is cultivated throughout Southeast Asia because of its multiple benefits to soil and human health. It is nutritionally important crop with about 25% protein nearly three times that of cereals, 60% carbohydrates, 1.3% fat (Das *et al.,* 2021) as well as important vitamins and minerals, making it a balanced vegan diet when supplemented with cereals. The ability of its roots to fix atmospheric nitrogen (42 kg/ha/year) (Dey *et al.,* 2020) contribute towards soil health while deep roots prevents soil erosion by binding soil particles. Short duration of blackgram makes it suitable for intercropping with corn or millet or rotation with cereals like rice or wheat (Muthusamy and Pandiyan, 2018), adding another benefit for farmer.

India is the largest producer as well as the consumer of pulses accounting for more than 70 per cent of global production. It ranks fourth in position after bengalgram, redgram and greengram cultivated in an area of about 4.14 M ha, with an annual production of 2.23 M t and with a productivity of 538 kg ha^{-1}. Andhra Pradesh is one of the major blackgram growing states of India with an area of 3.93 lakh hectares, with a production of 3.65 lakh tons and a productivity of 929 kg ha^{-1} (Directorate of Economics and Statistics, DAC & FW 2020-2021).

Though India is the largest producer of pulses, around 2-3 million tons of pulses are imported annually to fulfill the domestic consumption requirement as the current yield increase trends may not be sufficient in dealing with the growing demand (Singh *et al.,* 2022). Despite the economic and nutritional value of pulses including blackgram, the sluggish growth in production is due to lack of exploitable genetic variability, absence of location

specific suitable ideotypes for different cropping systems, poor harvest index and susceptibility to various biotic and abiotic stresses (Chippy *et al.*, 2021). Hence, for a major breakthrough in yield and productivity of blackgram, it is imperative to develop high yielding varieties with desirable traits.

To meet the nutritional requirement of ever growing population, a large number of blackgram varieties are released and notified every year in India. These improved varieties normally developed through hybridization between genetically similar parents which leads to the less amount of genetic variability among newly developed cultivars. This creates a lot of confusion in identification of varieties, moreover these varieties were utilizing on commercial scale unofficially. Hence, there is an immediate need to establish the genetic identity of the cultivars to prevent the unauthorized use of varieties on commercial scale.

For planning and execution of a successful breeding programme, the most essential prerequisite is the availability of substantial desirable genetic variability for important characters. The available variability in a population can be partitioned into genetic parameters such as coefficients of variation, heritability and genetic advance to serve as basis for selection of desirable genotypes than existing ones. The estimates of genotypic and phenotypic coefficient are necessary for understanding the influence of environment on different traits. Heritability measures the relative amount of the heritable portion of total variation and aids in phenotypic selection. Genetic advance (GA) under selection gives an idea about how much of the genetic gain possible in next generation due to simple selection alone. Estimation of genetic variability in conjunction with the estimates of heritability and genetic advance indicate the possible improvement achieved through selection (Johnson *et al.*, 1955a).

The pace and magnitude of genetic improvement are generally dependent on the amount of genetic diversity present in a population. The accurate estimation of genetic diversity can be invaluable in the selection of

diverse parental combinations to generate segregating progenies with maximum genetic variability. The selection of highly genetically divergent parents is expected to throw superior and desirable segregants following crossing (Bhatt, 1973). Several biometrical approaches have shown to be useful in selecting parents for successful hybridization programme. The Mahalanobis D^2 statistics has been found to be a powerful tool in quantifying the degree of genetic divergence among parents, which ensures high heterotic effects and more variability in the segregating generations and determines the relative contribution of each component character to the total divergence (Murthy and Arunachalam, 1966).

Yield being an intricate character, have low heritability due to its polygenic nature. Thus, progress from direct selection for such traits is slow. A better alternative is to consider the indirect selection through secondary traits that are relatively easier to measure and possess high heritability. Such traits can be identified based on analysis of relationship among traits. Correlation and regression analyses are the most popular statistics used for analysing trait relationships. However, in correlation and simple linear regression, relationship between only a pair of traits can be considered at a time. More recently, the genotype main effect plus genotype by environment interaction (GGE) biplot offers genotype by trait (GT) biplot analysis using robust statistical tools.

GT biplot analysis allows the visualization of genetic correlation among traits (Yan and Reid 2008) and also helps in studying genotype by trait relationships (Lee *et al*., 2003) and evaluation of genotypes on the basis of multiple traits. It facilitates the graphic visualization of the genetic correlations among traits (Yan and Rajcan, 2002; Lee *et al*., 2003) and it also provides information on the usefulness of genotypes for production and helps to detect less important (redundant) traits and identifies those that are appropriate for indirect selection for a target trait (Apraku and Akinwale 2010).

DNA fingerprinting is considered and widely used as potential tool for establishing genetic identity of the cultivars. Establishment of the genetic identity of the cultivars through development of cultivar specific molecular profiles is important for their registration under the Protection of Plant Varieties and Farmers Rights Act. Traditionally, morphological traits and isozyme markers were used to establish the identity of a variety (Glaszmann 1987; Patra and Chawla 2010). Such traits and markers are, due to their limited numbers, stage specificity and environmental influence of expression, not efficient enough in nature to characterize reliably and precisely. On the other hand, the highly heritable, abundant and polymorphic DNA markers are powerful enough to discriminate not only distantly related but also closely related genotypes.

Marker technology assist the conventional breeding by desirable gene identification, revealing the diversity of the genotypes, reducing the time of variety development and varietal identification (Latif *et al*., 2011). To date, so many markers such as RAPD (Williams *et al.*, 1990), AFLP (Vos *et al.*, 1995), SRAP (Li *et al.*, 2001), ISSR (Latif *et al.*, 2013), SSR (Latif *et al.*, 2013) and SNP (Monfared *et al.*, 2018) are developed for gene and varietal characterization of different crops. Among them, SSRs are the most popular molecular markers for its easy use, high allelic diversity, wide distribution across the genome, relative polymorphic abundance, amplification ability from low quantities DNA, cost effectiveness and reproducibility (Pathaichindachote *et al.*, 2019). SSRs are widely used for the characterization of several crops such as rice (Chakravarthi and Naravaneni., 2006), lentil (Agarwal and Katiyar., 2008), durian (Siew *et al*., 2018), groundnut (Amaravathi *et al*., 2014) and rice (Barghavi *et al*., 2021).

With the aim of improving the productivity of blackgram and protection of improved cultivars the present research work was formulated with the following objectives:

Objectives of Investigation:

1. To assess genetic variability and genetic parameters for yield and yield attributing traits.
2. To study genetic diversity among blackgram genotypes using Mahalanobis D^2 statistics.
3. To study the extent of association among yield and yield attributing traits by using genotype by trait biplot analysis.
4. Molecular profiling of selected blackgram genotypes using molecular markers.

Chapter - II

Review of Literature

Chapter II

REVIEW OF LITERATURE

An attempt has been made to review the literature in consonance with the objectives of present investigation in blackgram (*Vigna mungo* (L.) Hepper) under the following headings:

2.1 GENETIC VARIABILITY

2.2 GENETIC DIVERSITY

2.3 GENOTYPE BY TRAIT BIPLOT

2.4 MOLECULAR PROFILING

2.1 GENETIC VARIABILITY

Genetic improvement for quantitative traits depends on the nature and amount of variability present in genetic stock and the extent to which the desirable traits are heritable. Hence, prior to initiating any crop improvement program the knowledge of the inheritance of yield and its component traits through estimation of genetic variability parameters *viz.,* genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2), genetic advance (GA) and genetic advance as per cent of mean(GAM) is pre requisite.

A brief review of literature on variability, heritability and genetic advance of quantitative characters in blackgram is presented here under.

Deepshikha *et al.* (2014) assessed genetic variability in 30 blackgram genotypes and found that the phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all characters. PCV and GCV were high for grain yield per plant and number of pods per plant, while moderate for number of clusters per plant, number of seeds per pod and seed index.

Estimation of variability, heritability and genetic advance for eleven characters were carried out in 76 blackgram genotypes. High heritability was

observed for days to maturity, days to 50% flowering, grain yield per plant, number of pods per plant, number of clusters per plant, 100-seed weight and harvest index. Grain yield per plant, number of pod per plant and number of clusters per plant recorded high genetic advance coupled with high heritability (Patel and Patil, 2014).

Punia *et al.* (2014) conducted an experiment to estimate genetic parameters among 133 accessions of blackgram. Seed yield, branches per plant, pods per plant and plant height recorded high GCV and PCV respectively. Higher genetic advance coupled with high heritability was observed for plant height, seeds per pod, days to flowering and days to maturity.

Kumar *et al.* (2015) assessed variability in 57 blackgram genotypes for 14 quantitative characters and revealed that high heritability along with high genetic advance was recorded for plant height, pod weight, seed yield and total biomass, while high genetic advance along with moderate heritability recorded for pods per plant, seeds per plant and fodder biomass. Furthermore, moderate heritability with moderate genetic advance was observed for seeds per pod and harvest index.

Gowsalya *et al.* (2016) carried out genetic variability studies among 80 genotypes of blackgram for eleven quantitative characters and reported that the traits *viz.,* branch length, number of branches, plant height, clusters per plant, seed yield per plant, number of pods per plant and pod length registered high heritability. However, moderate heritability was recorded for 100 seed weight and number of seeds per pod.

Variability studies conducted by Priyanka *et al.* (2016) among 24 blackgram lines revealed the phenotypic coefficient of variation was greater than their corresponding genotypic coefficient of variation. The estimates for heritability was high for all the traits except for days to 50% flowering and branches per plant. High heritability coupled with high genetic advance as per cent of mean was noticed for the characters *viz.*, pods per cluster, pod length, test weight, seeds per pod and seed yield per plant.

In a study involving 36 blackgram genotypes by Anu *et al.* (2017) deciphered high estimates of PCV and GCV for number of clusters per plant, whereas moderate values registered for plant height and low for seed index, pods per plant, days to maturity and 100 seed weight.

Variability studies conducted by Hemalatha *et al.* (2017) among 25 blackgram genotypes revealed that the characters *viz.*, plant height, number of branches per plant, clusters per plant and pods per plant showed higher estimates of genetic advance as percent of mean, whereas the characters days to 50% flowering, seeds per pod and pod length showed moderate estimates of genetic advance as percent of mean.

Genetic variability in five breeding lines of blackgram for different quantitative characters was estimated. Among the characters studied, high GCV and PCVwere recorded for number of pods per plant and yield per plot, whereas moderate estimates of PCV and GCV observed for days to 50% flowering and days to maturity and Low for number of seeds per pod and 100 seed weight (Ozukum and Sharma (2017).

Panda *et al.* (2017) reported that high heritability estimates were registered for the traits *viz.*, number of clusters per plant, 100 seed weight, plant height, number of pods per plant, number of seeds per plant and seed yield per plant among 50 blackgram genotypes evaluated to estimate genetic variability. Moderate heritability was observed for days to maturity, days to 50% flowering, while the magnitude of heritability was low for number of seeds per pod, pod girth and pod length.

Aftab *et al.* (2018) estimated genetic parameters for thirteen characters in 39 blackgram genotypes. High heritability was recorded for seed yield per plant followed by biological yield per plant, plant height, harvest index, days to 50% pod setting, primary branches per plant, pods per plant, clusters per plant. the traits *viz.,* days to maturity, days to 50% flowering, 100-seed weight, pod length expressed moderate heritability.

Jeberson *et al.* (2018) conducted genetic variability studies in 25 advanced breeding lines of blackgram. Moderate estimates of heritability coupled with high genetic advance mean were recorded for seed yield per plant (18.39%), while moderate estimates of genetic advance coupled with high heritability was recorded for the 100 seed weight (16.02%) and plant height (13.57%).

Genetic variability analysis was carried out for seventeen yield, yield components and water use efficiency traits among 30 blackgram genotypes by Kavitha *et al.* (2018). They noticed that the characters *viz.*, seed yield per plant, number of clusters per plant, number of pods per plant, 100 seed weight, number of seeds per pod, number of primary branches per plant and SLA at 50 DAS exhibited high genetic advance as per cent of mean.

Priya *et al.* (2018) evaluated 20 blackgram genotypes for genetic variability and found that highest genetic variation was observed in number of primary branches per plant (GCV 29.1% and PCV 32.9%); number of clusters per plant (GCV 36.8% and PCV 38.3%); number of pods per plant (GCV 37.2% and PCV 38.0%) and single plant yield (GCV 33.9% and PCV 35.4%).

Thirty two blackgram genotypes were analyzed for genetic variabilityby Sushmitharaj *et al.* (2018) and stated that high heritability coupled with high genetic advance as per cent of mean was observed for the traits *viz.,* plant height, number of clusters per plant, number of pods per plant and single plant yield.

Forty five blackgram genotypes were subjected to genetic variability by Tank *et al.* (2018) and revealed that narrow difference was observed between GCV and PCV observed for characters *viz.,* 100 seed weight (GCV 13.67% and PCV 14.42%) followed by days to 75 percent maturity (GCV 2.72% and PCV 3.48%), seed protein content (GCV 4.67% and PCV 5.57%) and number of branches per plant (GCV 22.39% and 23.47%).

Thirty nine genotypes of blackgram were studied for genetic variability by Veni *et al.* (2018). They reported high GCV estimates for single plant yield, number of clusters per pod, plant height, number of branches per plant, hundred seed weight, number of seeds per pod and pod length. Among the characters, number of clusters per plant, number of pods per plant, hundred seed weight and single plant yield recorded high heritability coupled with high genetic advance.

An experiment was carried out to estimate the genetic parameters for 12 quantitative characters in 28 genotypes of blackgram by Chaitanya *et al.* (2019) and revealed that GCV and PCV were high for harvest index and seed yield per plant. High heritability coupled with moderate genetic advance was observed for number of pods per plant, pod length, number of primary branches per plant, harvest index, biological yield and seed yield per plant.

Partap *et al.* (2019) assessed 42 blackgram genotypes for 11 characters and reported that the PCV was higher in magnitude than the respective GCV for all the characters. The traits plant height followed by numberof pods per plant and number of primary branches per plant exhibited high PCV and GCV among all the traits.

Senthamizhselvi *et al.* (2019) assessed 112 blackgram genotypes to estimate genetic parameters. They noticed that plant height, number of primary branches per plant, number of clusters per plant, number of pods per plant, hundred seed weight, protein content and seed yield per plant recorded high heritability along with high genetic advance as percentage of mean. However, high heritability with low genetic advance is observed for the character days to 50% flowering.

Khan *et al*. (2020) studied genetic variability for fourteen traits in 60 genotypes of blackgram. They concluded maximum heritability was found for 100 seed weight (92%) followed by number of pods per plant (90%) and pod length (88%). Similarly, maximum genetic gain was observed for number of

pods per plant (48.17%), seed yield per plant (47.06 %) followed by cluster per plant (43.38 %) and harvest index (41.01%).

Variability studies conducted in 50 blackgram genotypes by Patel *et al.* (2020) divulged the higher magnitude of genotypic coefficient of variation was observed for plant height, clusters per plant, 100-seed weight, seed yield per plant and straw yield. High estimates of heritability coupled with high genetic advance expressed as percentage of mean was observed for branches per plant, clusters per plant, plant height, seed yield per plant, harvest index and straw yield.

Tushar *et al*. (2020) evaluated 51 released and advanced breeding lines of blackgram genotypes in RBD with three replications and stated that the estimates of GCV and PCV were high for number of branches per plant, number of pods per plant and seed yield per plant, while moderate GCV and PCV were observed for plant height, seeds per pod and pod length.

Evaluation of 35 accessions of blackgram by Saran *et al.* (2021) revealed highest genetic gain for seed yield per plant followed by plant height, 100-seed weight, harvest index and the number of pods per cluster. While number of branches per plant, pod length, the number of clusters per plant, the number of pods per plant, the number of seeds per pod and biological yield per plant recorded moderate genetic gain.

Aman *et al.* (2022) carried out genetic variability studies among 20 blackgram genotypes. High heritability coupled with high genetic advance as percentage of mean was observed for number of primary branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, pod length, number of seeds per pod, 1000 seed weight, seed yield per plant, biological yield per plant and harvest index.

2.2 GENETIC DIVERSITY

Genetic diversity is the basic requirement for successful breeding programme. The development of new varieties is mainly governed by the magnitude of genetic diversity in the base material and extent of variability for the desired characters. Progenies derived from a set of diverse crosses are expected to throw a broad spectrum of genetic variability and thereby providing a wide scope for isolating high yielding segregants in the advanced generations. Among the several techniques used for diversity analysis, Mahalanobis (1936) D^2 analysis was found to be the best technique in quantifying the degree of divergence among all possible pairs of population at genotypic level and therefore widely used for the classification of parental lines.

A brief review of literature on genetic diversity in blackgram is presented here under.

Leninkumar *et al.* (2014) studied multivariate analysis in 50 genotypes of blackgram and grouped them into eight clusters using D^2 analysis. Among the characters studied, number of seeds per plant (16.33%),number of clusters per plant (12.90%), seed yield per plant (8.41%), 50 per cent flowering (8.33%) and pods per plant (7.35%) were the major yield traits contributed towards genetic divergence among accessions.

Panigrahi *et al.* (2014) evaluated genetic diversity in 19 blackgram genotypes for ten quantitative traits and genotypes were grouped them into six distinct clusters. Among the yield attributing traits, the maximum contribution towards divergence was made by seed yield per plant (36.84%) followed by 100 seed weight (19.88%) and number of clusters per plant (11.69%).

Assessment of genetic diversity for eleven characters of blackgram has been worked out by Mahalanobis D^2 statistics. The 76 blackgram genotypes studied were grouped into fourteen clusters. Cluster III was largest with 32 genotypes followed by cluster I with 26 genotypes, cluster V with 7 genotypes

and all the remaining clusters consists of one genotype each (Patel and Patil, 2014).

Sarkar (2014) assessed the genetic diversity of 66 genotypes of blackgram by using D^2 statistic for various agro morphological traits and the genotypes were grouped into seven clusters having significant inter cluster distances. He noted that none of the clusters possessed a genotype which could be designated as superior for all traits.

Sixty four blackgram genotypes were analysed by Babu *et al. (*2015) to study the genetic divergence pattern in two seasons. The intra cluster D^2 values ranged from 0 (cluster VI and VII) to 7.68 (cluster IX), while the inter cluster D^2 values varied from 2.82 to 22.32. The lowest inter cluster distance was recorded between cluster I and II, while it was highest between clusters VI and IX.

Hadimani *et al.* (2016) conducted D^2 analysis among 64 blackgram genotypes and grouped them into 12 clusters. Inter cluster distance was highest between cluster IV and XII. Among the traits studied, plant height contributed maximum towards the divergence followed by days to 50% flowering, days to maturity, clusters per plant and 100 seed weight.

Kamannavar *et al.* (2016) were evaluated 144 blackgram germplasm accessions for genetic diversity and grouped into eight clusters following Mahalanobis D^2 statistics. Pods per plant (42.5%) followed by plant height (27.5%) and pod length (10.5%) contributed maximum towards the genetic diversity.

Gowsalya *et al.* (2017) studied 80 genotypes of blackgram comprising landraces, varieties and local cultivars from different locations for genetic divergence by using D^2 statistic. The genotypes were grouped into twelve clusters. The characters *viz.* number of branches, plant height, 100 seed weight and number of clusters per plant had maximum contribution to divergence.

Thirty two blackgram genotypes were evaluated for genetic divergence and revealed that the per cent contribution towards total genetic divergence was highest for seed index (29.44) followed by number of branches per plant (16.33), clusters per plant (16.13), pods per plant (10.08), biological yield per plant (8.67), harvest index (8.06) and days to 50 per cent flowering (3.83). (Mahesha and Gabriel, 2017).

Reddy *et al.* (2017) conducted an experiment to investigate the genetic diversity in 40 blackgram genotypes along with one check (T-9) and those were grouped into seven clusters by Tocher's method. The percent contribution of thirteen characters towards total genetic divergence was the highest for biological yield (65.26) followed by pod length (22.56), plant height (8.46) and seed yield per plot (2.05).

Genetic divergence analysis was carried out by using D^2 statistic from the means of 64 blackgram genotypes for thirteen characters. All the genotypes were grouped into 9 clusters. The intra and inter cluster D^2 values indicated that the inter cluster distances were greater than intra cluster distances which shows considerable amount of genetic diversity existed among the genotypes (Gopinath and Desai, 2018)

A set of 30 blackgram genotypes were assessed by Kavitha *et al.* (2018) for genetic diversity using Mahalanobis D^2 statistics and the genotypes were grouped into eight clusters. The maximum intercluster distance was observed between cluster III and VI. The maximum intracluster distance was reported in cluster V.

Priya *et al.* (2018) evaluated genetic diversity in 104 blackgram genotypes and grouped them into eight clusters. The inter cluster distance between clusters VI and VIII was found to be highest. Cluster V had low mean value for days to 50% flowering and cluster VII had high mean value for plant height, number of primary branches per plant.

Twenty two genotypes of blackgram were used to study the nature and magnitude of genetic divergence using Mahalanobis D^2 statistics by Vyas *et al.* (2018). The genotypes were grouped into five clusters. The average intra cluster distance between the genotypes was maximum in the cluster IV (49.2) followed by cluster V (23.98), III (17.86), I and II (1.00), respectively.

Eighty nine blackgram accessions were subjected to D^2 analysis by Bharathi *et al.* (2019) to assess the diversity for 18 quantitative traits. The genotypes were grouped into 14 clusters. The inter cluster distances were greater than intracluster distances, revealing that considerable amount of genetic diversity existed among the accessions. Maximum inter cluster distance was observed between cluster IX and X (815.75) followed by clusters III and X (752.52), cluster II and X (746.95) and cluster X and XII (654.83).

Jayashree *et al.* (2019) carried out genetic divergence analysis among 50 blackgram genotypes and grouped them into thirteen clusters. The cluster I was formed with sixteen genotypes followed by eight genotypes present in the cluster VII, five genotypes in cluster VIII, three genotypes in cluster VI and X, cluster XIII was mono genotypic and the remaining seven clusters were formed with two genotypes.

Forty six blackgram genotypes were evaluated by Panwar *et al.* (2019) to assess genetic diversity and grouped them into 12 clusters. The inter cluster distances were greater than intra cluster distances, revealing that considerable amount of genetic diversity existed among the genotypes of different clusters. Highest intra cluster distance was 2.148 observed for cluster I which comprises of five genotypes, whereas the maximum inter cluster distance (9.90) recorded between cluster V and cluster II.

Senthilkumar (2019) assessed nature and magnitude of genetic divergence in 35 blackgram genotypes. They were grouped into seven clusters and results revealed that the genotypes included in cluster IV were early flowing types (31.51) followed by cluster VI (33.46) and clutter II (33.47). Cluster VII recorded lowest mean for plant height (29.43 cm), maximum number of seeds

per plant (6.37) and seed yield per plant (13.05 g). Cluster II recorded (13.05g) highest number of branches per plant (4.57) and hundred seed weight (5.14 g). Cluster V recorded highest number of clusters per plant (12.60), number of pods per plant (35.57). Cluster IV recorded highest number of pods per cluster.

Narendrabhai *et al.* (2020) studied genetic diversity for thirteen traits in 50 blackgram genotypes by D^2 analysis during late kharif 2018. Based on D^2 values they grouped the genotypes into six clusters. From the cluster mean results, they concluded that cluster II, III and VI has high amount of variation within population.

Genetic divergence analysis was conducted among 40 diverse genotypes of blackgram by Pratap *et al.* (2020) and grouped them into 5 clusters. The results showed that the contribution of pod length was highest (12.70) followed by harvest index (11.06), number of pods per plant (10.81), test weight (10.68), days to maturity (10.56), grain yield per plant (9.86), plant height (8.86), biological yield per plant (6.80), number of seeds per pod (6.60), number of primary branches per plant (6.49) and lower contribution was made by days to 50% flowering (5.58) towards the genetic divergence.

Hundred blackgram genotypes were grouped into 14 clusters using D^2 analysis by Punithavathy *et al.* (2020). Maximum inter cluster distance (16.51) was observed between cluster IV and XI followed by cluster XI and XII (13.96). The lowest inter cluster distance was found between cluster III and VIII (4.45).

Rajalakshmi *et al.* (2020) assessed 32 blackgram genotypes for genetic diversity and grouped them into 9 discrete clusters. Among them, cluster I grouped with a maximum of 11 genotypes, followed by cluster IV with seven genotypes, clusters II and III each with four genotypes and cluster VIII with two genotypes, while the remaining clusters were solitary. They also found that the maximum contribution to genetic divergence was by the number of branches per plant followed by plant height and hundred seed weight.

Ayesha *et al.* (2021) evaluated genetic divergence of 40 blackgram genotypes. They were grouped in to 10 clusters. The distance within the clusters varied from 0.00 to 28.00. Cluster V recorded the maximum intra cluster distance of 28.00, followed by cluster IV (20.32), cluster IX (17.04), and cluster II (16.97).

Chippy *et al.* (2021) studied 102 blackgram genotypes for genetic diversity and grouped them into 18 distinct clusters. The cluster mean for the nine characters revealed that cluster X with one genotype (IC 436811) had the lowest mean value for days to 50 % flowering. The highest mean value for plant height (58.80 cm) was recorded in cluster XIII; cluster XVII for the number of pods per plant (48.80) and cluster XIV for the trait single plant yield (29.10 g).

Forty blackgram genotypes were grouped into 7 clusters using Mahalanobis D^2 statistic. The distribution of genotypes in different clusters revealed grouping of genotypes from different geographic regions into the same cluster. Similarly genotypes from the same geographic region were scattered into different clusters at random (Mallikarjuna *et al.*, 2021).

Reni *et al.* (2022) evaluated 59 blackgram genotypes for fourteen characters to assess genetic diversity. The genotypes were clustered in to seven clusters. Cluster I have highest number (24) of genotypes, followed by cluster II (23 genotypes), cluster III (7 genotypes) and cluster V (2 genotypes). The clusters IV, VI and VII were solitary with single genotype.

Forty six genotypes were subjected to genetic diversity studies by Goswami *et al.* (2022) and were grouped them into nine clusters. The inter cluster distances surpassed the intra cluster distances. Highest inter cluster distance was noted between cluster IV and VII followed by cluster VII and VIII, and cluster VI and VII.

Kuchanur *et al.* (2022) carried out genetic diversity analysis among 100 blackgram genotypes using Mahalanobis D^2 statistic. They found that the contribution of days to maturity towards genetic divergence was maximum

(66.04%). The inter cluster distance ranged from 15.50 to 514.44. The genotypes belongs to the cluster III, BDU-20, BDU-3-20, BDU-68, TRCRU-22 possessed desirable traits like earliness and higher seed size and genotypes of cluster IV, BDU-9, BDU-10, LBG-752 possessed traits like higher reproductive period, maturity and high seed yield were identified as diverse.

2.3 GENOTYPE BY TRAIT BIPLOT (GT BIPLOT)

The genotype by trait (GT) biplot analysis is a powerful statistical tool for studying relationships among traits, evaluating cultivars based on multiple traits and for identifying those that are superior in certain traits. A GT biplot can also be used to visualize the merits and shortcomings of individual genotypes, which is important for both cultivar evaluation and parent selection. It allows visual display of the genetic correlation among traits (Yan and Rajcan, 2002; Lee *et al.,* 2003) and provides information on the usefulness of cultivars for production as well as information that helps to detect less important traits and to identify those that are appropriate for indirect selection for a target trait.

A brief resume of the work done on genotype by trait biplot analysis in various crop plants is presented here under, as limited work has been done in blackgram.

Yan and Rajcan (2002) evaluated soybean cultivars to investigate the relationship between seed yield and four other traits by using GT biplot analysis. They revealed that a strong positive association between protein yield and oil yield and negative association between protein concentration and oil concentration. They reported a near zero correlation between seed yield and protein concentration and between seed yield and oil concentration.

Four winter rapeseed cultivars were assessed by Hamid *et al.* (2008) to understand the interrelationships among different traits using genotype by trait biplot. They identified SLM046 as the ideal cultivar for seed yield and oil content and also revealed that thousand seed weight was positively and significantly associated with seed yield per plant and number of pods per plant.

Akcura (2009) evaluated 42 bread wheat pure lines to investigate the relationships between yield and attributing traits using genotype trait (GT) biplot analysis. The results revealed a strong positive association between number of grains per spikelet, harvest index and biological yield. The genotypes *viz.,* G26 (Samsun-TR37926-3), G45 (Erzurum-TR32881-6) and G49 (Erzurum-TR32668-1) were identified as best parents for enhancing quality in bread wheat.

Aghaee *et al.* (2010) investigated 85 durum wheat accessions to study the relationship among 19 traits using accession by trait (AT) biplot analysis. The accession by trait biplot captured 36.20 % of the total variation of the standardized data. Based on their results, they revealed that AT biplot graphically displayed the interrelationships among traits and facilitated visual comparison of accessions.

Twenty three early maize inbreds were evaluated under artificial striga infestation and striga free conditions to identify the most appropriate traits for selecting striga resistant inbreds by Apraku *et al.* (2010) using GT biplot analysis. They identified TZEI 3, TZEI 2, TZEI 11 and TZEI 15 as potential inbred parents for the production of striga resistant hybrids and TZEI 8 was the earliest in maturity based on both GT biplot and multi trait selection tools. They further mentioned that the plant height and ear height were positively correlated with maturity.

Saeed *et al.* (2010) used genotype by trait biplot analysis to examine its usefulness in visualizing maize traits relationship and its application in hybrids comparison. Their results revealed a strong positive relationship between all the measured traits, except anthesis-silking interval.

Mohammadi *et al.* (2011) evaluated 18 durum wheat breeding lines along with two checks under three different climate locations and two moisture regimes by using GT biplot. Biplot analysis revealed that the locations tended to discriminate genotypes in dissimilar fashions and the relationships among traits were not consistent over the locations. Genotypes ch1/Brach//Mora,

Gcn//Stj/Mrb3 and 44-16-2-4 were identified as the highest yielding genotypes under moderate, warm and cold conditions, respectively.

Oladejo *et al.* (2011) assessed 30 cowpea cultivars to determine the interrelationship among physiological traits by using GT biplot. They identified that grain yield was positively correlated with days to 50% flowering, vigour index, pod weight, yield growth rate, pods per plant, yield per plant, seeds per pod, days to 50% physiological maturity, days to 50% podding and negatively correlated with flowering traits.

Twelve sugarcane genotypes were studied by Chatwachirawong *et al.* (2012) by using GT biplot. Their results revealed that cane yield was positively correlated with number of stalks, stalk height at six months after planting, stalk height at harvest and stalk weight, whereas cane yield was negatively correlated with brix value, pol value, fibre content and number of died tillers.

Safari *et al.* (2013) evaluated thirty nine peanut genotypes based on genotype by trait biplot analysis for simultaneous improvement of genotypes for multiple traits. They identified ICGV 93134 followed by ICGV 92118 and ICGV 93233 as the ideal cultivars.

Paramesh (2014) evaluated 31 mungbean genotypes using GT biplot analysis. Results based on GT biplot revealed that seed yield was highly positively correlated with all the morphological traits except 100 seed weight, which had approximately negative correlation with it. The traits *viz.,* chlorophyll content, specific leaf area, relative injury and chlorophyll stability index showed a positive correlation with seed yield among physiological and drought related traits.

Thirty six greengram genotypes were evaluated by Singh *et al.* (2014) for 17 yield component traits and studied trait associations using genotype by trait biplot analysis. They reported positive association of seed yield with days to 50% flowering, number of primary branches per plant, number of secondary branches per plant, number of clusters per plant, number of pods per cluster,

pod length, number of seeds per pod , pod mass, seed mass, seed index and harvest index.

Kamrani (2015) evaluated thirty bread wheat genotypes by using GT biplot analysis to study the effect of drought stress on the relationship among several agro-morphological traits. The results revealed a positive relationship among the traits *viz.,* the number of kernels per spike, chlorophyll content, flag leaf area, flag leaf weight, biological yield, days to maturity and grain yield in rainfed condition, whereas in irrigated conditions stress tolerance index, geometric mean productivity and mean productivity indices were positively correlated with grain yield.

Reddy *et al*. (2015) evaluated twenty four foxtail millet genotypes including three checks by using GT biplot method to understand the trait association and for selection of better genotypes. The GT biplot analysis exhibited 78.05 per cent of the total variation. They reported that grain yield was positively associated with panicle length, plant height, days to maturity, days to 50% flowering and number of tillers per plant and negatively associated with fodder yield.

Sofia *et al.* (2016) conducted genotype by trait biplot analysis among 35 greengram genotypes to understand the trait associations and noticed that the traits *viz.,* number of clusters per plant, number of pods per plant, days to maturity, number of pods per cluster, leaf area duration and chlorophyll content had positive correlation with seed yield per plant. In contrast, seed yield per plant was negatively correlated with 100 seed weight.

Sharma *et al.* (2018) observed positive correlation of plot yield with plant height, pods per plant, seeds per pod and pod length based on GT biplot analysis among 60 blackgram genotypes. Based on GT biplot based graphical representation of association between traits, they revealed that the traits *viz.,* plant height, pods per plant, seeds per pod and pod length showed positive association with yield.

A study on association among 21 agronomic traits in 19 maize genotypes using GT biplot analysis revealed a strong positive correlation between grain yield and nutrient use efficiency, 100 kernel weight, number of ears per plant, plant height, number of kernels per plant, number of kernels per row, grain nitrogen utilization efficiency and grain nitrogen content (Naggar *et al.* 2020).

Shojaei *et al.* (2020) examined trait relationships among twelve maize hybrids using GT biplot analysis. They identified that the genotypes *viz.*, KSC705, KSC706 and SC647 exhibited superior performance for maximum yield traits and identified a significant positive correlation among ear length, number of rows in ear and grain width.

Forty six blackgram genotypes were studied by Kavitha *et al.* (2021) using GT Biplot. The traits *viz.*, number of pods per cluster, harvest index, pod length, number of pods per plant, number of primary branches per plant, number of clusters per plant, number of seeds per pod, 100- seed weight and plant height showed positive association with seed yield per plant. However, seed yield per plant was negatively associated with YMV scores, days to 50% flowering and days to maturity. Based on the biplot, LBG-623 was found as ideal cultivar for plant height followed by MBG-1058, TBG-104, MBG-1050 and MBG-1037.

2.4 MOLECULAR PROFILING

Conservation of the genetic resources of blackgram is vital for future breeding programs and food security. For this characterization and proper assignation of individual genotypes to species is required. Molecular profiling is an effective method for varietal identification. DNA banding pattern developed by using different primers for each genotype serves as its genetic identity, which is more precise, rapid and distinct. Among various DNA fingerprinting techniques, Simple Sequence Repeat (SSR) markers were mostly used by many of the scientists to identify cultivars of various crops because they are multi allelic, abundant, randomly and widely distributed throughout

the genome, co-dominant that could differentiate plants with homozygous or heterozygous alleles, simple to assay, highly reliable, reproducible.

A brief resume of the work done on molecular profiling in various crop plants using SSR markers is presented here under, as limited work has been done in blackgram.

DNA fingerprinting of 15 elite rice genotypes using 30 SSR primers on chromosome numbers 7-12 and revealed all the primers showed distinct polymorphism among the cultivars studied. The information obtained from the DNA fingerprinting studies helped to distinctly identify and characterize nine varieties using 18 different RM primers (Chakravarthi and Naravaneni, 2006).

A set of 31 lentil genotypes having indigenous and exotic origin were screened using 42 STMS markers. Out of the 42 STMS primers tested, all of them gave amplified products. Among them 36 (85.7%) primers were found to be polymorphic. A set of 16 STMS markers have been identified which could differentiate all the lentil cultivars of India. (Agarwal and Katiyar, 2008)

Sarao *et al.* (2010) fingerprinted 14 rice varieties using 75 simple sequence repeat (SSR) primers. Out of these, 58 primers produced polymorphic profiles, while 13 were monomorphic, two revealed null allele and the remaining two amplified only from super basmati. They reported that in a screen of seven cultivars, 16 SSR loci produced 17 rare/unique alleles, which provided an opportunity for their unambiguous identification.

Kuang *et al.* (2011) constructed a DNA fingerprinting database of 32 cotton major cultivars based on simple sequence repeats (SSR) method using 36 primers. Among the 32 varieties, 36 primer pairs had 142 polymorphic bands and 3.94 alleles were detected by each SSR primer pair on an average with the range from 2 to 11. Ten cultivars had specific alleles by 9 primer pairs. Thirty two major cultivars could be identified by 5 primer combinations at least.

Ninety eight microsatellite markers were amplified in 15 accessions of sugarcane by Silva *et al.* (2012). The three SSR loci out of 98 primers amplified a total of 31 alleles in fifteen genotypes, with size ranging from 192 to 505 bp and the number of alleles ranging from 2 to 8 per individual, showing the polyploid nature of sugarcane. The PIC average value among the SSR primers was 0.821, with a minimum of 0.752 (SCC93) and maximum of 0.875 (SCC82).

Amaravathi *et al.* (2014) fingerprinted a set of 12 groundnut varieties employing SSR markers. A total of 300 SSR were screened and fifteen potential markers were employed for fingerprinting of groundnut varieties. The SSR markers generated alleles ranging from 2 to 7 with an average of four per locus. The polymorphism information content (PIC) values ranged from 0 to 0.85. They found a set of highly informative primers *viz.*, PM 377, TC1A02, TC5A06 and GM1489 has the potential to identify most of the groundnut varieties.

Kaewwongwal *et al*. (2015) assessed 520 cultivated and 14 wild accessions of blackgram using 22 SSR markers. Totally, 199 alleles were detected, with the number of alleles per locus ranging from 3 (cp01038 and VES0019) to 23 (CEDG305 and cp05325) and a mean of 9.05 alleles per locus. The PIC values varied from 0.02 (CEDG015) to 0.92 (CEDG305 and cp05325) with an average of 0.60.

A total of 30 blackgram genotypes including five locally adapted varieties, twenty three advanced breeding lines and two local germplasm were evaluated using 12 SSR markers by Pyngrope *et al*. (2015) and revealed that the most informative loci were CEDG 180, CEDG 139 and CEDG 279 with 5 alleles, CEDG 006, CEDG 143 and CEDG 056 with 4 alleles and CEDG 282, CEDG 204, CEDG 118, CEDG 068, CEDG 008, CEDG 043 with 3 alleles each.

Fifty eight jute accessions, including two control varieties (Huangma 179 and Kuanyechangguo) were evaluated with 28 pairs of SSR primers. A total of 184 polymorphic loci were identified. Each primer detected 3 to 15 polymorphic loci, with an average of 6.6. These markers differentiated the 58 jute accessions from one another, with CoSSR305-120 and CoSSR174-195

differentiating Huangma 179 and Kuanyechangguo, respectively (Zhang *et al*., 2015).

Fifty four wheat genotypes comprising of 41 Indian origin and 13 exotic genotypes were characterized using 39 polymorphic SSR markers for DNA fingerprinting. A total of 112 alleles ranging from one to seven alleles were generated with an average of 2.87 per SSR marker. The PIC value of SSR markers ranged from 0.03 to 0.49. Furthermore, 7 SSR markers generated alleles specific to some of the wheat genotypes (Sundeep *et al*., 2016).

Reflinur *et al*. (2017) studied total of 22 Indonesian mungbean accessions based on 21 morphological traits and 55 simple sequence repeats (SSRs) primers. They identified eight multi allele markers with high polymorphic information content (PIC) values which have been successfully selected for varietal identification. The genetic identity of a variety was shown by digital barcoding which represented a series of alleles produced by corresponding markers.

Seventeen mustard cultivars were subjected to SSR assay to identify the efficient primers for developing DNA fingerprinting. Two SSR primers of mustard Ra1H11 and Ra1F03 exhibited a considerable DNA polymorphism of 41.17% and 35.29% respectively. The SSR primer Ra1H11 (0.772) of mustard had showed highest discriminating power followed by Ra1F09 (0.617) then Ra1F03 (0.558), Ra2B02 (0.382) and Ra1G07 (0.228) respectively. The highest PIC value had showed by Ra1F03 (0.525), then followed by Ra1H11 (0.363), Ra2B02 (0.359), Ra1F09 (0.290) respectively. Among all the mustard SSR primers Ra1H11, Ra1F09, Ra1F03 followed a descending order of their efficiency to discriminate the mustard cultivars and those could be utilised for developing DNA finger printing patterns for mustard. (Panigrahi *et al.,* 2018).

A total of 25 SSR primers were used by Ragul *et al.* (2018) to differentiate VBN 3 from Vamban 1 and CO(CP) 7. Out of 25 primers, six primers *viz.*, CEDG156, CP09781, CEDG171, CEDG127, CEDG008 and CEDG 305 had polymorphism among varieties. Primers *viz.,* CEDG156,

CP09781, CEDG171 and CEDG008 had polymorphism between Vamban 1 and VBN 3. Likewise, primers CEDG 156, CP09781, CEDG127 and CEDG305 had polymorphism between VBN 3 and CO(CP) 7. One primer, CP09781 was able to differentiate all three varieties.

Siew *et al.* (2018) used simple sequence repeat (SSR) markers to study the genetic variation in 27 durian types. Of the eight SSR primer pairs designed, seven primer pairs successfully amplified clear and reproducible bands in all 27 durian types. Out of seven primers five loci were polymorphic and two were monomorphic. A total of 19 alleles were scored across seven SSR loci, ranging from one to five alleles per locus with an average of 2.714 alleles per locus. The allele frequency of each allele at each locus ranged from 0.074 to 1. Unique DNA fingerprints were generated for 21 out of 27 durian types using five polymorphic SSR markers.

Eighteen mungbean genotypes was assessed using a panel of 40 microsatellite based primer pairs by Suman *et al.* (2019). They identified that out of 40 primers initially tested, only 24 showed distinct polymorphism and consequently only these primers were utilized for the purpose of genome profiling of the entries. The polymorphic information content (PIC) ranged from 0.440 to 0.928 with an average of 0.822 per SSR primer pair. The lowest polymorphism percent was observed for primer CEDGAG001 (16.67), while the primer CEDG 154 gave the maximum polymorphism percent (63.64). The primer pairs CEDG 008, CEDG 068 and CEDG 154 among the total primer pairs were found to be highly informative.

A total of 66 alleles were identified from 60 blackgram genotypes evaluated using 11 SSR markers by Suvan *et al*. (2020). The number of alleles per SSR marker was six with a range of 2–12. PIC value ranged from 0.30 (CEDG 92) to 0.90 (Vmg SSR 29 and Vmg SSR 53) with average PIC of 0.60. The major allelic frequency ranged from 0.13 to 0.78.

Baisakh *et al.* (2021) studied 47 blackgram genotypes for genetic variation based on molecular markers and morpho economic traits. They

noticed a higher level of polymorphism (97.05%) with high average PIC (polymorphic information content) value (0.75). Eight hundred and forty bp allele (band) characteristic to Kantapada local - A, Kendrapada local-D and Nayagarh local – C was identified.

Chapter - III

Material and Methods

Chapter III

MATERIAL AND METHODS

The details of the material utilized, methodologies adopted and statistical analysis followed in the present investigation entitled “GT biplot analysis for yield traits and molecular profiling of selected lines in blackgram [*Vigna mungo* (L.) Hepper]” are briefly presented in this chapter under the following headings

3.1 MATERIAL

The experimental material consisted of 35 genotypes of blackgram. The material was obtained for the study from Agricultural Research Station (ARS) Madhira, Telangana; Regional Agricultural Research Station (RARS), Lam, Guntur and Regional Agricultural Research Station (RARS), Tirupati of Andhra Pradesh. The list of genotypes was furnished in Table 3.1.

3.2 METHODS

3.2.1 Location of The Experimental Site

The present experiment was carried out at dry land farm of Sri Venkateswara Agricultural College, Tirupati, ANGRAU situated at an altitude of 182.9 m above mean sea level (MSL), 32.27°N latitude and 79.36°E longitude, geographically in southern agro climatic zone of Andhra Pradesh. The soil is sandy loam with medium fertility.

3.2.2 Field Layout

The field was ploughed and harrowed twice until a fine tilth of soil was obtained. The experiment was laid out in a Randomized Block Design (RBD) with three replications. The crop was sown on 29th October 2021 and each genotype was sown in three rows of 3 m length with a spacing of 30 cm between rows and 10 cm between plants within rows.

3.2.3 Crop Husbandry

Thinning was done at seedling stage to leave single seedling per hill. The crop was fertilized at the rate of 20 kg N and 40 kg P_2 in the form of urea and single super phosphate. Irrigation, weeding and plant protection measures were taken up as and when needed during the crop growth period, as per the standard recommended package of practices of ANGRAU.

3.2.4 Observations Recorded

Observations were recorded on five randomly chosen competitive plants from the central row in each genotype in each replication for all the characters except days to 50 per cent flowering and days to maturity. The latter two characters were recorded per plot basis. The values of five competitive plants were averaged and expressed as mean of the respective characters. The details of data recording are as follows.

3.2.4.1 Days to 50 per cent flowering (days)

The number of days taken from the date of sowing to the date on which 50 per cent plants in each row reached to flowering was taken as days to 50 per cent flowering.

3.2.4.2 Days to maturity (days)

The number of days taken from the date of sowing to the 80 per cent physiological maturity of pods in each genotype from each plot was considered as days to maturity for that genotype.

3.2.4.3 Plant height (cm)

At the time of harvest, plant height was measured from ground level to the tip of the main raceme. The mean value of sample plants was recorded as plant height in centimeters.

Plate. 1. Field view of the experimental plot

Table 3.1. List of 35 blackgram genotypes used in the present study

S. No.	Genotypes	Source
1	KDRS-136	IIPR, Kanpur
2	SB-25-19	AAU, Shillongani
3	PU-205	GBPUAT, Pantnagar
4	COBG-653	TNAU, Coimbatore
5	P-726	IIPR, Kanpur
6	VBG-11-6	Vamban, Tamilnadu
7	VBN-7	Vamban, Tamilnadu
8	SB-40-5	AAU, Shillongani
9	TBG-104	Tirupati, Andhra Pradesh
10	NDU-11-204	NDUA and T, Faizabad
11	IPU-10-4	IIPR, Kanpur
12	P-1032	IIPR, Kanpur
13	MBG-1058	Madhira, Telangana
14	PU-31	GBPUAT, Pantnagar
15	VBG-10-010	Vamban, Tamilnadu
16	MBG-1061	Madhira, Telangana
17	UG-708	IIPR, Kanpur
18	VBN-4	Vamban, Tamilnadu
19	MBG-1051	Madhira, Telangana
20	TU-94-2	Coimbatore, Tamilnadu
21	P-728	IIPR, Kanpur
22	WBG-26	Warangal, Telangana
23	LBG-22	Lam, Guntur
24	LBG-787	Lam, Guntur
25	LBG-752	Lam, Guntur
26	TU-67	BARC, Mumbai
27	MBG-223	Madhira, Telangana
28	P-112	IIPR, Kanpur
29	LBG-709	Lam, Guntur
30	MBG-1050	Madhira, Telangana
31	RVSU-60	Sehore
32	LBG-648	Lam, Guntur
33	LBG-645	Lam, Guntur
34	LBG-20	Lam, Guntur
35	VBG-11-31	Vamban, Tamilnadu

3.2.4.4 Number of primary branches per plant

The number of primary branches originating from the main axis were counted on each sample plant from each entry and mean value of sample plants was recorded as the number of primary branches per plant.

3.2.4.5 Number of clusters per plant

At the time of harvest, the number of pod bearing clusters were counted on each sample plant and mean of all the sample plants was recorded as the number of clusters per plant.

3.2.4.6 Number of pods per cluster

The number of pods in each cluster from each sample plant were counted and the average was calculated.

3.2.4.7 Number of pods per plant

The number of well filled pods from each sample plant was counted and the mean of the random sample plants was recorded as the number of pods per plant.

3.2.4.8 Number of seeds per pod

Randomly five pods from each sample plant were selected, threshed separately and average number of seeds per pod was calculated.

3.2.4.9 Pod length (cm)

The length of the pod was measured from the base to the tip of the pod on five randomly selected pods from each sample plant at harvest. The mean value of the sample was recorded as the length of pod in centimeters.

3.2.4.10 Hundred seed weight (g)

From each sample plant at random one hundred seeds were counted and weighed in grams. The average of the sample plants were expressed as 100 seed weight.

3.2.4.11 Seed yield per plant (g)

The weight of seeds of each of five sampled plants was measured in grams and mean value was recorded as seed yield per plant.

3.2.4.12 Harvest index (%)

It is calculated as the ratio of seed yield (economic yield) to total dry weight (biological yield) in grams and expressed in per cent as given below.

$$HI = \frac{\text{Economic yield per plant(g)}}{\text{Biological yield per plant(g)}} \times 100$$

3.2.5 Statistical Analysis

3.2.5.1 Analysis of variance

The analysis of variance for each character was calculated as per the standard statistical procedure given by Panse and Sukhatme (1961).

$$Y_{ij} = \mu + g_i + \gamma_j + e_{ij}$$

Where,

Y_{ij} = Phenotypic observation on 'i'th genotype in 'j'th replication.

μ = General mean

g_i = Effect of ith genotype

γ_j = Effect of jth replication

e_{ij} = Random error associated with ith genotype in jth replication.

The analysis of variance for each character was carried out as follows:

ANOVA for Randomized Complete Block Design

Source of variation	**Degrees of freedom**	**Mean Sum of Squares**	**Expected Mean Sum of Squares**	**'F' calculated Value**
Replications	(r-1)	M_r	$\sigma^2e + g\,\sigma^2r$	M_r/M_e
Genotypes	(g-1)	M_g	$\sigma^2e + r\,\sigma^2g$	Mg/M_e
Error	(r-1) (g-1)	M_e	σ^2e	
Total	(rg-1)			

Where,

r = Number of replications

g = Number of genotypes

M_r = Mean sum of square due to replication

M_g = Mean sum of square due to genotype

M_e = Mean sum of square due to error

σ^2_e = Environmental variance

σ^2_r = Variance due to replications

σ^2_g = Variance due to genotypes

Test of significance for each character was carried out against the corresponding error degrees of freedom using 'F' table values given by Fisher and Yates (1967).

3.2.5.2 Estimation of genetic parameters

3.2.5.2.1 Variance

The genotypic and phenotypic variances were calculated as per the formulae proposed by Burton (1952)

$$\text{Genotypic variance } \left(\sigma_e^2\right) = \frac{\text{MSS due to genotypes - MSS due to error}}{\text{Number of replications}}$$

$$\text{Phenotypic variance } \left(\sigma_p^2\right) = \sigma_g^2 + \sigma_e^2$$

σ_g^2 = Genotypic variance

σ_e^2 = Error variance

3.2.5.2.2 Genotypic and phenotypic coefficient of variation

The genotypic (GCV) and phenotypic (PCV) coefficient of variation were computed by the formulae given by Burton (1952).

$$GCV\ (\%) = \frac{\sigma_g}{\bar{X}} \times 100$$

$$PCV\ (\%) = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviation

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

Categorization of the range of variation was done as proposed by Sivasubramanian and Madhavamenon (1973)

Less than 10 % - Low

10 – 20 % - Moderate

More than 20 % - High

3.2.5.2.3 Heritability (Broad sense)

The proportion of genotypic variance to the total variance of the population is referred to as heritability in broad sense [$h^2_{(b)}$] and was calculated by the formula given by Lush (1940).

$$Heritability = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

$h^2_{(b)}$= Heritability in broad sense

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance $\left(\sigma_g^2 + \sigma_e^2\right)$

σ_e^2 = Environmental variance

As suggested by Johnson *et al.* (1955b), heritability estimates were categorized as

Less than 30 % - Low

30 – 60 % - Moderate

More than 60 % - High

3.2.5.2.4 Genetic advance

Genetic advance refers to the expected genetic gain or improvement in the next generation by selecting the superior individuals under certain amount of selection pressure. From the heritability estimates, the genetic advance was estimated by the following formula given by Johnson *et al.* (1955a).

$$GA = k\sigma_p H$$

Where,

GA = Genetic advance

k = Selection differential at 5% selection intensity (2.06)

σ_p = Phenotypic standard deviation

H = Heritability (broad sense)

3.2.5.2.5 Genetic advance as per cent of mean (GAM)

Genetic advance as per cent of mean was calculated as per the formula.

$$GAM = \frac{GA}{\overline{X}} \times 100$$

Where,

GA = Genetic advance

$\overline{X}$ = Grand mean of the character

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

Less than 10% - Low

10 – 20 % - Moderate

More than 20 % - High

3.2.5.3 Genetic divergence analysis:

3.2.5.3.1 Mahalanobis D^2 analysis

The data collected on different characters was analyzed using Mahalanobis D^2 analysis to determine the genetic divergence among the genotypes.

3.2.5.3.1.1 Test of significance

Variances were calculated for all the characters investigated and test of significance was done. Analysis of covariance for the character pairs was estimated based on mean values (Panse and Sukhatme, 1961). After testing the difference between genotypes for each of the characters, a simultaneous test of significance for differences in the mean values of a number of correlated variables with regard to the pooled effect of characters was carried out using 'V' statistic, which in turn utilizes Wilk's criterion (Wilks, 1932). The sum of squares and sum of products of error and error + variety, variance – covariance matrix were used for this purpose.

The estimation of Wilk's criterion was done using the following relationship.

$$\lambda = \frac{(E)}{(E+V)}$$

Where,

λ = Wilk's criterion

(E) = Determinant of error matrix and

(E+V) = Determinant of error + variety matrix

The significance of 'λ' was tested by

$$\chi^2_{pq} = V = - m \log_e \lambda$$

Where,

m = n - (p + q + 1)/2 with 'pq' degree of freedom

n = Degrees of freedom of error + varieties

p = Number of characters

q = Number of genotypes – 1

$\log_e \lambda = 2.3407 \log_{10} \lambda$

V (Stat) is distributed as χ^2 with pq degrees of freedom.

3.2.5.3.1.2 Transformation of correlated variables

Transformation was done using pivotal condensation method. Transformation of correlated variables into standardized uncorrelated ones was done before working out the D^2 values because computation of D^2 values was reduced to simple enumeration of differences in mean values of various characters of the two genotypes *i.e.,* Σdi^2.

3.2.5.3.1.3 Computation of D^2 values

The D^2 value between 'i^{th}' and 'j^{th}' genotypes for 'p' characters was calculated as

$$D_{ij}^2 = p\Sigma_{t=1} (\overline{Y_{it}} - \overline{Y_{jt}})^2$$

Where,

$\overline{Y_{it}}$ *is* uncorrelated mean value of i^{th} genotype for character 't'

$\overline{Y_{jt}}$ is uncorrelated mean value of j^{th} genotype for character 't'

D_{ij}^2 is D^2 between i^{th} and j^{th} genotype.

3.2.5.3.1.4 Testing the significance of D^2 values

The D^2 value obtained for a pair of genotypes is taken as calculated value of χ^2 and is tested against the tabulated value of χ^2 for p degrees of freedom where 'p' is the number of characters considered.

3.2.5.3.1.5 Grouping of genotypes into various clusters

The grouping of genotypes into different clusters was done using the Tocher's method as described by Rao (1952). The criterion was that, two varieties belonging to the same cluster at least on an average show a smaller D^2 value than those belonging to different clusters. For this purpose D^2 values of all combinations of each genotype were arranged in ascending order of magnitude in a tabular form as described by Singh and Choudhary (1977).

To start with two genotypes having the closest distance from each other were considered, to which the third genotype having the smallest D^2 value from the first two genotypes was considered and so on. Similarly, the next nearest fourth population was considered and this procedure was continued. At certain stage when it was felt that after adding a particular genotype there was an abrupt increase in the average D^2, that the genotype was not considered for including in that cluster. The genotypes of the first cluster were then eliminated and the rest were treated in a similar way. This procedure was continued till all the genotypes were included into one or other cluster.

3.2.5.3.1.6 Average intra cluster distance

For the measurement of intra cluster distances, the formula used was

$$\frac{\Sigma D^2 \mathrm{i}}{n}$$

Where,

$\Sigma D^2 i$ = The sum of distances between all possible combinations (n) of populations included in a cluster.

3.2.5.3.1.7 Average inter cluster distance

Clusters were taken one by one and the distances from other clusters were calculated. The distance between two clusters was the sum of D^2 values between the members of one cluster to each of the members of the other clusters divided by the product of number of genotypes in both the clusters under consideration.

$$\text{Average inter cluster distance} = \frac{D^2}{(n_1 \times n_2)}$$

Where, n_1 and n_2 are number of genotypes of two clusters.

3.2.5.3.1.8 Cluster diagram

The clusters and their mutual relationships were presented diagrammatically. The square root of average D^2, which was an approximate measure of divergence between groups, had been used to denote the distance.

3.2.5.3.1.9 Contribution of individual characters towards divergence

In all combinations, each character was ranked on the basis of their contribution towards divergence between two entries ($d_i = Y_{it} - Y_{jt}$). Rank 1 is given to the highest mean difference and the rank P to the lowest difference, where P is the total number of characters. Percentage contribution of each character (X) towards genetic divergence was calculated using the following formula.

$$\text{Percentage contribution of the character X} = \frac{(N \times 100)}{M}$$

Where,

N = Number of genotype combinations where the character was ranked first

M = All possible combinations of number of genotypic pairs

3.2.5.4 Genotype by trait biplot (GT biplot) analysis

The Genotype by Trait (GT) biplot approach (Yan and Rajcan, 2002) was used to display the genotype by trait data in a biplot and is based on the parental means expressed in the following formula

$$\frac{\alpha_{ij} - \beta_j}{\sigma_j} = \sum_{n=1}^{2} \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} = \sum_{n=1}^{2} \xi_{in} \eta^*_{jn} + \varepsilon_{ij}$$

Where,

α_{ij} = The mean value of genotype i for trait j

β_j = The mean value of all genotypes for trait j

σ_j = The standard deviation of trait j among genotype means

λ_n = The singular value for Principal Component (PCn)

ξ_{in} = The PCn score for genotype i

η_{jn} = The PCn score for trait j

ε_{ij} = The residual associated with genotype i in trait j

To achieve trait focused scaling between genotype and trait scores the singular value λ_n has to be absorbed by the singular vector for genotype ξ_{in} and for traits η_{jn}. That is, $\xi^*_{in} = \xi_{in} \lambda^0_n = \xi_{in}$ and $\eta^*_{jn} = \eta_{jn} \lambda^1_n = \eta_{jn} \lambda_n$. Because n=2 in a biplot, only PC1 and PC2 are retained in the model and such a model tends to be best for extracting patterns and rejecting noise from the data. The Genotype by Trait (GT) biplot is constructed using ‘‘R’’ packages (version 3.1.1) by plotting PC1 scores against PC2 scores for each genotype and each trait.

3.2.6 Molecular Profiling Using SSR Markers

The DNA was isolated from 35 blackgram genotypes using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1990).

3.2.6.1 List of equipments and chemicals used

Equipments and chemicals used for molecular profiling are listed in the Appendix A and B respectively.

3.2.6.2 DNA isolation

The total genomic DNA was isolated from tender leaves by modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method (Doyle and Doyle, l990) with the following steps.

1. The leaf samples (healthy leaves were collected from young plants of about 10 days old) were grounded into fine powder with liquid nitrogen.
2. Approximately 100 mg of the grinded leaf powder was transferred into 2 ml eppendorf tube by spatula, to this 1.5 ml of CTAB extraction buffer (Appendix C) was added and then incubated in water bath at 65 °C for 45 min with occasional mixing at every 20 min interval.
3. The tubes were removed from the water bath and allowed to cool at room temperature.
4. Then equal volume of Phenol:Chloroform (1:1 v/v) was added and mixed thoroughly by gentle inversion and centrifuged for 20 min by keeping in rotator at 13000 rpm (Eppendorf centrifuge, 5804R) at 25 °C until clear separation of three layers was attained.
5. Again Phenol: Chloroform (1:1v/v) step repeated for complete separation of polysaccharides and then the supernatant was carefully pipetted out into new 2 ml eppendorf tubes.
6. To this supernatant Chloroform: Iso amyl alcohol (24:1 v/v) was added and mixed thoroughly by gentle inversion and centrifuged at 12000 rpm for 15 min at 25 °C until clear separation of layers was attained. Then supernatant was carefully pipetted out into new 1.5 ml eppendorf tubes.
7. To the supernatant, ice cold isopropanol of about 0.6 volumes (2/3rd of pipetted volume) and 50 µl of 3 M sodium acetate of pH 5.2 (Appendix C) was added. The contents were mixed gently by inversion and kept undisturbed for about 2 hours at -20 °C.
8. Subsequently, the tubes were centrifuged at 13000 rpm for 20 min at 4 °C temperature to pellet out DNA.
9. The supernatant was discarded gently and the DNA pellet was washed with 70 % ethanol and centrifuged at 13000 rpm for 10 min.

10. The supernatant was removed and the tubes were allowed to air dry completely until ethanol smell was lost and then the pellet was dissolved in 50 µl of milli-Q water.

11. The dissolved DNA was stored in -20 °C after labeling.

3.2.6.3 Assessment of quality and quantity of DNA

DNA was assessed for its purity and intactness using both agarose gel and Nano Drop spectrophotometer.

3.2.6.3.1 Quantification of DNA by 0.8 % agarose gel electrophoresis:

3.2.6.3.1.1 Preparation of 0.8 % agarose gel:

A conical flask containing 100 ml 1X TBE buffer was taken and 0.8 g of agarose was added to it (Appendix C). The conical flask along with its contents were placed in an oven until agarose gets melted completely and clear solution was formed and then the flask was taken out from the oven and allowed to cool. To this 100 ml of agarose gel, 3 µl of ethidium bromide (10 mg ml^{-1}) was added and mixed thoroughly. Later the solution was poured slowly into the gel casting tray which is preset with 0.5 mm combs without formation of bubbles. After solidification, the gel with casting tray was placed in gel tank and the comb was removed gently without disturbing the wells that formed upon solidification.

3.2.6.3.1.2 Electrophoresis of DNA samples:

Three micro litre of each of dissolved genomic DNA samples were mixed with 2 µl of 6X Gel loading dye (Appendix C) and were loaded in the 0.8 % agarose-1X TBE gel along with lambda (λ) Hind III digest (New England Biolabs, UK). Then, the gel with loaded samples was electrophoresed at 90V at room temperature for about an hour. After that, the gel was visualized in an UV gel documentation system (Biorad Gel Doc XR^{+} Imaging System) and saved the image for further use. Later, based on the intensity and thickness of genomic DNA compared to λ DNA, the concentration of individual samples DNA was determined.

3.2.6.3.2 Quantification of DNA by nanodrop spectrophotometer

The isolated DNA was quantified using Nanodrop (ND-1000, Thermo Scientific, Nanodrop Technologies, U.S.A).

1. The upper and lower pedestals of lifted sampling arm of Nanodrop were wiped by using sterilized distilled water and 2 µl of distilled water was loaded into hole located on the lower pedestal.
2. The upper arm was closed and the operating software in the attached computer was initiated and the instrument was calibrated to zero. Then sampling arm was lifted and loaded water was wiped out by paper towel.
3. Then 1 µl of genomic DNA sample was loaded in the same manner and the reading was noted under the 260/280 nm spectral ratio and the concentration. After taking the readings, both the arms of the instrument were soft wiped by a clean paper towel. Then DNA was diluted to 50 ng μl^{-1} based on the reading provided by the machine.
4. The ratio of OD_{260} / OD_{280} provides an estimate of purity of nucleic acid. Pure DNA preparations have OD_{260} / OD_{280} value of 1.8-2.0.
5. After quantification, all the samples were diluted to 50 ng μl^{-1} and used for PCR reactions.

The ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA. A ratio of approximately 1.8 is generally accepted as pure for DNA, a ratio of approximately 2.0 is generally accepted as pure for RNA. If the ratio is lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at/or near 280 nm.

3.2.6.4 Primers used for molecular profiling

A total of 16 SSR (Simple Sequence Repeats) markers were used for molecular profiling of 35 blackgram genotypes.

The list of markers was presented in the Table 3.2.

Table 3.2. List of SSR markers used for molecular profiling of 35 blackgram genotypes

S.NO	MARKER NAME	FORWARD PRIMER (5-3)	REVERSE PRIMER (3-5)	Ann.Temp (^{0}C)
1	CEDG024	CATCTTCCTCACCTGCATTC	TTTGGTGAAGATGACAGCCC	57
2	CEDG225	GAGGAAGTGTTGCAGCACC	GTAGACTCTGCAGAGGGATG	57
3	CEDG133	GCATACATAATGTGGTGAGATG	GTCTCGTGCCTTTCACAC	54
4	CEDG020	TATCCATACCCAGCTCAAGG	GCCATACCAAGAAAGAGG	54
5	CEDG044	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG	59
6	CEDG198	CAAGGAAGATGGAGAGAATC	CCTTCTAAGAACAGTGACATG	52
7	CEDG139	CAACTTCCGATCGAAAGCGCTTG	GTTTCTCTCCTCAATCTCAAGCTCCG	55
8	CEDG006	AATTGCTCTCGAACCAGCTC	GGTGTACAAGTGTGTGCAAG	57
9	CEDG173	GATAAGAGATGCATCACTC	CTTCTCTTCCATCACATCTG	49
10	BM170	AGCCAGGTGCAAGACCTTAG	AGATAGGGAGCTGGTGGTAGC	58
11	CEDAAG002	GCAGCAACGCACAGTTTCATGG	GCAAAACTTTTCACCGGTACGACC	66
12	CEDG282	CAGCAACAAGACATGGAGTG	GGTGACCACTTAGACAGAC	52
13	CEDG243	GACAACTCATCCATTCTTGAG	CCTATGGGATAGTGATACAGC	57
14	CEDG180	GGTATGGAGCAAAACAATC	GTGCGTGAAGTTGTCTTATC	55
15	CEDG178	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	59
16	CEDG172	GCTGACGTAGGTGACAACC	CGGCTTGTGCTTCATTGTCTG	59

3.2.6.5 Amplification of DNA using polymerase chain reaction

DNA was subjected to Polymerase Chain Reaction (PCR) by using SSR markers. PCR tubes of 0.2 ml were taken and 2 µl of DNA (100 ng μl^{-1}) was added. PCR reaction was performed in a 10 µl volume of mix containing the components as given below.

Components, their concentration and volume used for the polymerase chain reaction

Component	Concentration	Reaction volume
Taq buffer (10X) with $MgCl_2$	1X	1.0 µl
dNTP mix	10 mM	1.0 µl
Taq DNA polymerase	5U μl^{-1}	0.1 µl
Forward primer	10 pmol	0.5 µl
Reverse primer	10 pmol	0.5µl
Genomic DNA	100 ng μl^{-1}	2.0 µl
Autoclaved millipore water	-	4.9 µl

The reaction mixture was given a short spin for thorough mixing of components and then the PCR tubes with reaction mix were placed in the gradient thermal cycler (Eppendorf) and the reaction programme was set as shown below.

Steps used in the PCR Program

S.No	Steps	Temperature (˚C)	Time	Cycle number
1	Initial denaturation	94	5 min	1
2	Denaturation	94	1 min	35
3	Annealing	57-62	45 sec	
4	Extension	72	1 min	
5	Final extension	72	10 min	
6	Final hold	4	∞	1

The steps from 2-4 were repeated for 35 cycles for amplification of targeted DNA. Annealing temperature of each primer was standardized by doing PCR with the temperature range of 54-64 ˚C. The PCR products were stored at 4 ˚C for short periods and at -20 ˚C for long duration.

3.2.6.6 Resolution of the PCR products

Three per cent agarose gel was prepared by adding 3 g of agarose to 100 ml 1 X TBE buffer and boiled carefully till the agarose completely melted. Just before complete cooling 3 µ1 ethidium bromide (10 mg ml^{-1}) was added and the gel was poured in the tray containing the comb carefully avoiding formation of air bubbles. The solidified gel was transferred to horizontal electrophoresis apparatus and 1X TAE buffer was added to immerse the gel.

The PCR product was loaded on the 3 % agarose gel by mixing with 2 µl of 6X loading dye. A 50 bp ladder was loaded as a standard reference marker. The gel was run at constant voltage of 90 V for about 2-3 hours, until the ladder got properly separated. The banding pattern was analyzed using gel documentation system (Biorad Gel Doc XR^{+} Imaging Systems) and saved the image for later use. Reproducibility of PCR assay was tested by performing duplicate reactions at different times by using identical genotypes and primer combinations and only reproducible bands was recorded.

3.2.6.7 SSR data analysis

The amplified products for marker analysis were scored visually based on the presence (taken as ‘1’) or absence (taken as ‘0’) of band for each primer. Each marker fragment was treated as a unit character and only clear and unambiguous bands were scored. Also, the alleles were assigned band size relative to the molecular size ladder. Unique alleles were defined as those detected in only one species.

3.2.6.8 Marker polymorphism

To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR marker was calculated using GeneCalc

(Bio-informatics tool). PIC score of > 0.50 depicts that the marker is highly informative. Markers with PIC scores in between 0.25 – 0.50 are moderately informative, whereas PIC score of < 0.25 brings the marker under the category of least informative one.

3.2.6.9 QR codes

QR (Quick Response) codes were generated using online tool (available at www.barcode-generator.org) for 35 blackgram genotypes.

Chapter - IV

Results & Discussion

Chapter IV

RESULTS AND DISCUSSION

Thirty five blackgram genotypes were evaluated for variability, genetic divergence and genotype by trait biplot analysis for twelve quantitative traits *viz.*, days to 50% flowering, days to maturity, plant height, number of primary branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length, hundred seed weight, seed yield per plant and harvest index during *rabi* 2021-22 and molecular profiling was carried out for the same genotypes by using SSR markers.

The data collected on these traits were utilized for biometrical studies. The results obtained from these investigations are furnished here under.

4.1 ANALYSIS OF VARIANCE (ANOVA)

The analysis of variance revealed the significant differences among the genotypes for all the traits under study indicating the existence of sufficient variability in the material (Table 4.1).

4.2 MEAN PERFORMANCE

The mean performance of 35 blackgram genotypes are presented trait wise in Table 4.2.

4.2.1 Days to 50% Flowering

Days to 50% flowering ranged from 33.67 to 41.00 days. Among all the genotypes, VBG-10-010 (33.67 days) was the earliest to flower, whereas LBG-648 and LBG-645 were late in flowering (41.00 days). Nineteen genotypes were earlier in flowering when compared to the general mean (36.97 days).

Table 4.1. Analysis of variance for yield and yield attributing traits in 35 blackgram genotypes

S. No	Characters	Mean sum of squares		
		Replications	Genotypes	Error
		(df=2)	(df=34)	(df=68)
1	Days to 50 % flowering	1.37	10.03**	0.46
2	Days to maturity	2.66	24.38**	1.38
3	Plant height (cm)	0.86	78.48**	17.45
4	Number of primary branches per plant	0.28	0.92**	0.09
5	Number of clusters per plant	2.39	3.01**	0.78
6	Number of pods per cluster	0.36	0.33**	0.16
7	Number of pods per plant	34.36	25.93**	11.73
8	Number of seeds per pod	0.17	0.77**	0.09
9	Pod length (cm)	0.08	0.25**	0.04
10	100 seed weight (g)	0.52	0.67**	0.18
11	Seed yield per plant (g)	0.65	1.82**	0.41
12	Harvest index (%)	4.45	87.58**	43.79

** : Significant at 1% level.

Table 4.2. Mean performance of 35 blackgram genotypes for yield and yield attributing traits

S.NO	GENOTYPES	DFF	DM	PH	NPB	NCP	NPC	NPP	NSP	PL	100 SW	SYP	HI
1	KDRS-136	35.33	71.33	26.93	4.27	7.20	4.20	23.27	6.73	5.24	5.32	5.24	36.27
2	SB-25-19	35.33	69.33	22.23	3.80	5.53	4.40	17.40	6.13	4.50	5.39	4.23	49.13
3	PU-205	36.67	69.00	18.42	3.93	5.47	4.27	19.27	5.67	4.25	4.74	4.07	45.32
4	COBG-653	35.33	68.33	18.83	3.10	5.53	4.60	18.87	6.07	4.23	4.52	5.40	40.67
5	P-726	36.67	68.67	20.23	3.80	7.10	4.00	20.53	5.80	4.56	5.18	5.44	42.72
6	VBG-11-6	35.33	69.33	23.93	4.07	6.40	3.93	18.47	6.27	4.93	5.43	4.54	38.24
7	VBN-7	37.33	72.33	20.90	3.67	4.67	4.13	16.07	5.87	4.61	5.15	4.22	43.49
8	SB-40-5	36.33	70.00	20.70	3.67	4.80	4.07	17.47	6.13	4.39	5.17	5.07	41.04
9	TBG-104	36.33	70.33	21.80	3.50	5.73	4.47	19.67	6.47	4.75	5.38	5.11	37.86
10	NDU-11-204	38.33	78.33	26.45	3.63	5.53	4.27	19.40	6.13	4.82	5.70	5.24	39.26
11	IPU-10-4	36.33	71.67	17.97	1.73	6.03	4.73	25.67	5.60	4.25	5.15	5.19	42.97
12	P-1032	38.00	68.67	20.73	4.33	5.80	4.00	19.87	5.87	4.98	5.42	6.14	35.66
13	MBG-1058	37.00	73.00	24.13	4.60	7.67	4.80	28.33	6.60	4.91	5.16	5.44	36.59
14	PU-31	34.33	69.67	22.87	3.87	7.07	4.07	20.47	6.47	4.59	5.46	5.71	40.75
15	VBG-10-010	33.67	72.33	18.33	3.93	5.33	4.00	18.93	6.13	4.57	4.62	4.28	38.32
16	MBG-1061	38.67	74.33	26.23	3.07	4.93	4.27	17.00	6.13	4.88	5.86	4.07	28.91
17	UG-708	35.67	68.67	21.37	3.80	6.13	4.33	18.07	5.87	4.46	5.12	4.88	44.77
18	VBN-4	39.00	71.67	20.77	3.80	4.27	4.00	12.87	5.73	4.45	5.63	3.67	32.43
19	MBG-1051	39.67	73.33	26.33	3.80	5.93	4.40	16.27	6.80	5.31	6.40	4.18	28.90
20	TU-94-2	35.33	70.00	24.30	3.47	7.67	4.60	20.67	6.60	4.56	5.72	5.79	39.76
21	P-728	36.00	72.00	21.80	3.47	7.00	4.20	19.27	6.00	4.60	5.75	3.78	38.40
22	WBG-26	37.67	72.00	22.97	2.73	5.27	3.67	17.20	6.27	4.65	5.24	4.04	39.83

Cont.

Table 4.2. (Contd.).

S.NO	GENOTYPES	DFF	DM	PH	NPB	NCP	NPC	NPP	NSP	PL	100 SW	SYP	HI
23	LBG-22	39.67	72.67	25.10	3.57	5.80	3.87	20.40	5.27	4.57	5.65	5.21	38.83
24	LBG-787	37.00	73.00	24.17	2.70	6.00	3.93	16.87	5.53	4.75	5.85	3.87	29.93
25	LBG-752	34.67	71.33	24.43	3.07	5.27	3.87	16.60	6.93	4.72	5.56	4.00	34.43
26	TU-67	38.33	69.67	23.03	3.47	4.07	4.27	16.33	6.07	4.53	4.81	3.50	48.53
27	MBG-223	36.67	69.00	19.80	2.73	4.00	4.33	14.67	6.33	4.99	5.79	3.50	34.86
28	P-112	34.33	68.33	20.30	3.07	5.67	4.40	21.80	5.33	4.44	4.31	4.90	42.80
29	LBG-709	37.67	72.67	23.30	3.90	6.13	4.00	17.33	6.67	4.95	6.02	4.65	34.09
30	MBG-1050	39.33	72.67	23.77	3.93	5.33	4.27	18.47	6.40	4.89	5.50	4.13	34.32
31	RVSU-60	36.67	70.67	24.20	3.93	6.27	4.07	21.80	5.27	4.67	5.53	5.01	34.02
32	LBG-648	41.00	80.33	41.25	4.13	7.00	3.07	17.93	6.80	5.36	5.41	5.03	24.56
33	LBG-645	41.00	78.00	41.77	3.93	7.60	3.67	21.67	6.93	5.05	4.83	6.43	35.78
34	LBG-20	37.00	71.67	27.77	3.67	6.93	3.87	20.07	5.20	4.53	6.19	5.77	38.08
35	VBG-11-31	36.33	68.67	22.83	3.60	4.87	4.40	17.53	5.27	4.97	4.71	3.94	37.22
	General Mean	36.97	71.51	23.71	3.59	5.89	4.15	19.04	6.10	4.71	5.36	4.73	37.96
	Minimum	33.67	68.33	17.97	1.73	4.00	3.07	12.87	5.20	4.23	4.31	3.50	24.56
	Maximum	41.00	80.33	41.77	4.60	7.67	4.80	28.33	6.93	5.36	6.40	6.43	49.13
	C.D. 5%	1.10	1.92	6.81	0.49	1.44	0.65	5.58	0.48	0.33	0.70	1.04	10.78
	S.E. (d)	0.55	0.96	3.41	0.25	0.72	0.33	2.79	0.24	0.16	0.35	0.52	5.40
	S.E. (m)	0.39	0.67	2.37	0.17	0.50	0.23	1.94	0.17	0.11	0.24	0.36	3.77
	C.V. (%)	1.83	1.64	17.62	8.38	15.03	9.66	17.98	4.86	4.27	8.00	13.52	17.43

DFF: Days to 50 % flowering, **DM:** Days to maturity, **PH:** Plant height (cm), **NPBP:** Number of primary branches per plant, **NCP:** Number of clusters per plant, **NPC:** Number of pods per cluster, **NPP:** Number of pods per plant, **PL:** Pod length (cm), N**SP:** Number of seeds per pod, **100 SW:**100 seed weight (g), **SYP:** Seed yield per plant (g) and **HI:** Harvest index (%)

4.2.2 Days to Maturity

The trait number of days to maturity varied from 68.33 to 80.33 days. The genotypes P-112 and COBG-653 were early in maturity (68.33 days), while LBG-648 was found to be late in maturity (80.33 days). Eighteen genotypes were early in maturity when compared with the mean maturity (71.51 days) of the genotypes.

4.2.3 Plant Height (cm)

The shortest plant height ranged was registered by IPU-10-4 (17.97 cm), while the tallest was recorded by LBG-645 (41.77cm). Sixteen genotypes were taller in height when compared to the general mean height (23.71 cm).

4.2.4 Number of Primary branches per Plant

Number of primary branches expressed in a range from 1.73 to 4.60. Among all the genotypes, more number of primary branches per plant was recorded by the genotype MBG-1058 (4.60) whereas the lesser number was registered by IPU-10-4 (1.73). A greater number of primary branches per plant than the general mean of the genotypes (3.59) was shown by 22 genotypes.

4.2.5 Number of Clusters per Plant

Maximum number of clusters per plant was recorded by the genotypes MBG-1058 and TU-94-2 (7.67), while it was minimum in MBG-223 (4.00). Sixteen genotypes surpassed the general mean (5.89) of the genotypes.

4.2.6 Number of Pods per Cluster

The difference for this trait was between 3.07 to 4.80. Among the genotypes, more or less number of pods per cluster was registered by MBG-1058 (4.80) and LBG-648 (3.07). Eighteen genotypes showed greater number of pods per cluster than the general mean of the genotypes (4.15).

4.2.7 Number of Pods per Plant

Number of pods per plant ranged from 12.87 to 28.33. MBG-1058 (28.33) registered maximum number of pods per plant, whereas VBN-4

recorded a minimum (12.87) number of pods per plant. Sixteen genotypes excelled the general mean (19.04) of this trait.

4.2.8 Number of Seeds per Pod

Number of seeds per pod was highest in the genotypes LBG-645 (6.93), LBG-752 (6.93) and lowest in LBG-20 (5.20). Nineteen genotypes exhibited greater number of seeds per pod when compared to the general mean (6.10).

4.2.9 Pod Length (cm)

The genotype, COBG-653 (4.23 cm) registered the shortest pod length while LBG-648 (5.36 cm) was found to be the longest. Fifteen genotypes were longer in length when compared to the general mean (4.71 cm).

4.2.10 Hundred Seed Weight (g)

Among 35 genotypes, MBG-1051 recorded the maximum 100 seed weight (6.40 g), while P-112 had the minimum 100 seed weight (4.31 g). The general mean of 5.36 g was exceeded by 20 genotypes for 100 seed weight.

4.2.11 Seed Yield per Plant (g)

Seed yield per plant ranged from 3.50 g to 6.43 g with a general mean of 4.73 g. Out of 35 genotypes tested, LBG-645 recorded the highest seed yield (6.43 g), whereas MBG-223 and TU-67 registered the lowest seed yield per plant (3.50 g). Eighteen genotypes put forth higher seed yield per plant than general mean.

4.2.12 Harvest Index (%)

The maximum and minimum harvest index was observed in SB-25-19 (49.13%.) and LBG-648 (24.56%) respectively. Nineteen out of 35 genotypes were found to be exhibit higher harvest index than that of the general mean (37.96%).

In any plant breeding programme for developing high yielding varieties or hybrids the basic need is the choice of parents with high mean values as they are expected to produce desirable segregants upon crossing (Gilbert,

1958). Hence, selection of the genotypes with high *per se* performance are to be identified first for each of the traits under study, aimed towards development of high yielding blackgram varieties.

A perusal of mean values in the present investigation revealed that the genotypes P-112 followed by COBG-653, SB-25-19, UG-708 and VBG-11-6 were earliest to flower as well as earliest to mature. These genotypes may provide useful genetic variability in breeding programs aimed at developing short duration blackgram varieties.

Considering the plant height, the genotypes LBG-645 was the tallest followed by LBG-648, LBG-20, KDRS-136 and NDU-11-204. Similarly, higher number of primary branches per plant registered for the genotypes MBG-1058, P-1032, KDRS-136, LBG-648 and VBG-11-6. The genotypes with tall height and more primary branches per plant would produce increased number of clusters and number of pods per plant thereby seed yield per plant will be increased. Hence, these genotypes could be utilized in future breeding programme for improving these traits.

In blackgram, the seed yield mainly depends on the contribution of yield determining characters and among them, number of clusters per plant, number of pods per plant, number of pods per cluster and number of seeds per pod are very important. Plant types with more number of above said traits usually produce higher yields. In the present study, the genotypes TU-94-2, MBG-1058, LBG-645, KDRS-136, and P-726 registered more number of clusters per plant. Similarly, the genotypes MBG-1058, IPU-10-4, TU-94-2, COBG-653 and TBG-104 for number of pods per cluster; the genotypes MBG-1058, IPU-10-4, KDRS-136, P-112 and RVSU-60 for number of pods per plant and the genotypes LBG-645, LBG-752, LBG-648, MBG-1051 and KDRS-136 for number of seeds per pod registered superior performance. Hence, these genotypes have the maximum potential for utilization in hybridization programme to improve the seed yield in blackgram.

Hundred seed weight is one of the important attributes among the seed yield components as it has greater contribution towards seed yield. Higher test weight was registered by MBG-1051 followed by LBG-20, LBG-709, MBG-1061 and LBG-787. Hence, these genotypes could be utilized in future breeding programme for improving this trait.

Higher yield potential of genotypes is mainly attributed to increased biomass coupled with enhanced harvest index. The genotype SB-25-19 recorded the highest *per se* for harvest index followed by TU-67, PU-205, VBN-7, UG-708. Hence, these genotypes could be exploited for higher yield coupled with higher harvest index.

The ultimate aim of any breeding programme is to improve the yield. Hence, selection of high yielding genotypes plays an important role in improving the productivity of blackgram. Among the genotypes, LBG-645 recorded high mean for seed yield per plant followed by P-1032, TU-94-2, LBG-20 and PU-31 suggesting that these genotypes might be successfully exploited for blackgram yield improvement.

Based on the overall mean performance of genotypes , several genotypes showed high mean performance for more than one traits. Among the genotypes, LBG-645 and MBG-1058 registered high *per se* for seven yield attributes *viz.*, plant height, number of seeds per pod, number of primary branches per plant, number of clusters per plant and number of pods per plant, pod length and seed yield per plant. The next best genotype was TU-94-2 as it showed high *per se* for six yield traits *viz.*, number of clusters per plant, number of pods per plant, number of seeds per pod, 100 seed weight and seed yield per plant. hence, these genotypes could be utilized for the development of high yielding blackgram varieties. Further, PU-31 exhibited high *per se* for five yield and yield attributing traits *viz.*, number of clusters per plant, number of pods per plant, number of seeds per pod, seed yield per plant, harvest index and low *per se* for days to 50 per cent flowering. Similarly, P-1032 showed high *per se* for three yield and its attributing traits *viz.*, number of primary branches per plant, pod length, seed yield per plant and low *per se* for days to

maturity Hence, these genotypes could be well exploited as donors for development of short duration blackgram genotypes along with high yield.

From the foregoing discussion based on *per se*, it could be concluded that the genotypes LBG-645, MBG-1058 and TU-94-2 were adjudged as the best genotypes for improving yield, while the genotypes PU-31 and P-1032 for improving yield and yield attributes coupled with early maturing traits.

4.3 GENETIC PARAMETERS

4.3.1 Variability

The success of any breeding programme depends upon the extent of genetic variability in base population and it is essential to subject a population for selection to achieve improvement in a particular trait. The variability estimates as phenotypic and genotypic coefficients of variation, heritability in broad sense, genetic advance and genetic advance as per cent of mean for twelve characters in 35 blackgram genotypes are furnished in Table 4.3.

The estimates of variances due to genotypic, phenotypic and environmental effects for 12 quantitative traits exhibited wide variation among the traits. The higher estimates of genotypic and phenotypic variances were recorded for plant height (20.34 and 37.79) followed by harvest index (14.60 and 58.38) and number of pods per plant (4.74 and 16.46). In contrast, the traits number of pods per cluster (0.06 and 0.22), pod length (0.07 and 0.11), 100 seed weight (0.16 and 0.35), number of seeds per pod (0.23 and 0.31) and number of primary branches per plant (0.28 and 0.37) noted lower genotypic and phenotypic variances.

Moderate estimates of GCV and higher estimates of PCV were observed for the traits plant height (PCV: 25.93%; GCV: 19.02%), number of pods per plant (PCV: 21.31%; GCV: 11.43%), number of clusters per plant (PCV: 20.99%; GCV: 14.65%;) and harvest index (PCV: 20.13%; GCV: 10.06%). Similar pattern of the result was reported in blackgram by Patel *et al.* (2014) for number of clusters per plant; Veni *et al.* 2018 for plant height and Saran *et al.* (2021) for harvest index.

Table 4.3. Estimates of mean, range and genetic parameters for yield and yield attributing traits in 35 blackgram genotypes

S. No.	Characters	Mean	Range		Variance		Coefficient of Variation		Heritability (Broad sense) (%)	Genetic advance (GA)	Genetic advance as percent of mean (%)
			Min.	Max.	Genotypic	Phenotypic	Genotypic	Phenotypic			
1	Days to 50 % flowering	36.97	33.67	41.00	3.19	3.65	4.83	5.17	87.40	3.44	9.30
2	Days to maturity	71.51	68.33	80.33	7.67	9.05	3.87	4.21	84.72	5.25	7.34
3	Plant height (cm)	23.71	17.97	41.77	20.34	37.79	19.02	25.93	53.83	6.82	28.75
4	Number of primary branches per plant	3.59	1.73	4.60	0.28	0.37	14.60	16.83	75.22	0.94	26.08
5	Number of clusters per plant	5.89	4.00	7.67	0.74	1.53	14.65	20.99	48.70	1.24	21.06
6	Number of pods per cluster	4.15	3.07	4.80	0.06	0.22	5.75	11.25	26.16	0.25	6.06
7	Number of pods per plant	19.04	12.87	28.33	4.74	16.46	11.43	21.31	28.77	2.41	12.63
8	Number of seeds per pod	6.10	5.20	6.93	0.23	0.31	7.81	9.20	72.13	0.83	13.67
9	Pod length (cm)	4.71	4.23	5.36	0.07	0.11	5.60	7.04	63.21	0.43	9.17
10	100 seed weight (g)	5.36	4.31	6.40	0.16	0.35	7.49	10.96	46.77	0.57	10.56
11	Seed yield per plant (g)	4.73	3.50	6.43	0.47	0.88	14.49	19.82	53.47	1.03	21.83
12	Harvest index (%)	37.96	24.56	49.13	14.60	58.38	10.06	20.13	25.00	3.94	10.37

Moderate estimates of PCV and GCV were observed for the traits seed yield per plant (PCV: 19.82%; GCV: 14.49%) and number of primary branches per plant (PCV: 16.83%; GCV: 14.60%). These findings were in consonance with the findings of Patel *et al.* (2014) and Saran *et al.* (2021) for number of primary branches per plant; Kumar *et al.* (2015) and Jaberson *et al.* (2018) for seed yield per plant.

Moderate PCV and low GCV were observed for the character number of pods per cluster (PCV: 11.25%; GCV: 5.75%) and 100 seed weight (PCV: 10.96%; GCV: 7.49%). However, low estimates of co efficient of variation was observed for the characters number of seeds per pod (PCV: 9.20%; GCV: 7.81%), pod length (PCV: 7.04%; GCV: 5.60%), days to 50% flowering (PCV: 5.17%; GCV: 4.83%) and days to maturity (PCV: 4.21%; GCV:3.87%). Similar kind of findings were also reported by Tank *et al.* (2018) and Khan *et al.* (2020) for days to 50% flowering and days to maturity; Jaberson *et al.* (2018) for 100 seed weight; Veni *et al.* (2018) for days to 50% flowering and pod length and Senthamizhselvi *et al.* (2019) for days to 50% flowering, number of seeds per pod and pod length.

4.3.2 Heritability (Broad Sense)

Heritability measures the relative amount of heritable portion of variability. It is a good index of the transmission of characters from parents to offspring. It is important selection parameter and provides clues on possible improvement. Lush (1949) gave the concept of broad sense heritability, which is the ratio of genotypic variance to total or phenotypic variance. It is the most important aspect of genetic constitution of breeding material, determines the degree of success in a selection programme. In the present study heritability in broad sense was estimated and presented in Table 4.3.

The highest heritability was registered for days to 50% flowering (87.40%) followed by days to maturity (84.72%), number of primary branches per plant (75.22%), number of seeds per pod (72.13%) and pod length (63.21%) in the decreasing order of their magnitude indicating the least

influence of environment on these characters. This was in line with the studies by Tank *et al.* (2018) and Saran *et al.* (2021) for days to maturity, number of primary branches per plant, number of seeds per pod and pod length; Priya *et al.* (2018) and Senthamizhselvi *et al.* (2019) for days to 50% flowering and number of primary branches per plant; Sathees *et al.* (2019) for days to 50% flowering and pod length and Aman *et al.* (2022) for number of primary branches per plant.

Moderate heritability was registered for plant height (53.83%) followed by seed yield per plant (53.47%), number of clusters per plant (48.70%) and 100 seed weight (46.77%). These results were in agreement with the findings of Gowsalya *et al.* (2016) for 100 seed weight; Tank *et al.* (2018) for seed yield per plant; Aftab *et al.* (2018) for 100 seed weight; Jaberson *et al.* (2018) for plant height, seed yield per plant and number of clusters per plant and Veni *et al.* (2018) for plant height; In contrary, low heritability was registered for number of pods per plant (28.77%), number of pods per cluster (26.16%) and harvest index (25.00%). These results were in agreement with the findings of Kumar *et al.* (2015) for harvest index and Gill *et al.* (2017) for number of pods per plant.

4.3.3 Genetic Advance

Knowledge of heritability coupled with genetic advance is most useful in predicting the scope for genetic improvement through selection. Selection made on the basis of heritability alone is likely to be misleading. Since, broad sense heritability includes both additive and epistatic effect, and it will be reliable only when heritability coupled with genetic advance. Thus, selection of traits based on heritability and genetic advance estimates is of great importance to the breeder for making criteria for improvement in a complex character.

The traits *viz.*, plant height (6.82), days to maturity (5.25), harvest index (3.94), days to 50% flowering(3.44), number of pods per plant (2.41), number of clusters per plant (1.24), seed yield per plant (1.03), number of

primary branches per plant (0.94), number of seeds per pod (0.83) 100 seed weight (0.57), pod length (0.43) and number of pods per cluster (0.25) registered low estimates of genetic advance in the decreasing order and indicated that most of the traits were controlled by polygenes.

4.3.4 Genetic Advance as Per cent of Mean

In the present study, the maximum genetic advance as per cent of mean was registered for plant height (28.75%) followed by number of primary branches per plant (26.08%), seed yield per plant (21.83%) and number of clusters per plant (21.06%). Similar results were reported by Veni *et al.* (2018) and Sathees *et al.* (2019) for number of primary branches per plant, seed yield per plant and number of clusters per plant; Senthamizhselvi *et al.* (2019) and Saran *et al.* (2021) for plant height, number of primary branches per plant, seed yield per plant and number of clusters per plant; Khan *et al.* (2020) for plant height, seed yield per plant and number of clusters per plant and Aman *et al.* (2022) for number of primary branches per plant.

The traits, number of seeds per pod (13.67%), number of pods per plant (12.63%), 100 seed weight (10.56) and harvest index (10.37%) recorded moderate genetic advance as per cent of mean. These findings were in consonance with Tank *et al.* (2018) and Aftab *et al.* (2018) for harvest index; Priya *et al.* (2018) for number of seeds per pod and 100 seed weight; Jaberson *et al.* (2018) for 100 seed weight; Veni *et al.* (2018), Sathees *et al.* (2019) and Khan *et al.* (2020) for number of seeds per pod and Saran *et al.* (2021) for number of pods per plant.

On contrary, the traits *viz.,* days to 50 per cent flowering (9.30%), pod length (9.17%), days to maturity (7.34%) and number of pods per cluster (6.06) showed low genetic advance as per cent of mean. These findings were in consonance with Tank *et al.* (2018) and Aftab *et al.* (2018) for days to 50 per cent flowering and days to maturity : Priya *et al.* (2018) for pod length and Veni *et al.* (2018) for days to 50 per cent flowering and pod length.

The boosting of yield and other yield related attributes may be achieved by efficient selection based on heritability and genetic advance estimations and recommended that direct selection based on these traits would be beneficial for crop improvement.

In the present investigation, high heritability coupled with high genetic advance as per cent of mean was recorded for number of primary branches per plant. Similarly, high heritability coupled with moderate genetic advance as per cent of mean was recorded for the character number of seeds per pod. High heritability coupled with low genetic advance as per cent of mean was recorded for days to 50% flowering, days to maturity and pod length. These results were in agreement with the findings of Tank *et al.* (2018) for number of primary branches per plant, number of seeds per pod and days to maturity; Aftab *et al.* (2018) for days to 50% flowering and days to maturity; Priya *et al.* (2018) and Senthamizhselvi *et al.* (2019) for number of primary branches per plant; Khan *et al.* (2020) for number of seeds per pod; Rehman *et al.* (2021) for pod length; Saran *et al.* (2021) for number of primary branches per plant and days to maturity; Aman *et al.* (2022) for number of primary branches per plant.

Moderate heritability coupled with high genetic advance as per cent of mean was recorded for plant height, number of clusters per plant and seed yield per plant. Sowmini and Jayamani (2013) and Hemalatha *et al.* (2017) found the similar result in blackgram for plant height; Jaberson *et al.* (2018) for number of clusters per plant and Tank *et al* (2018) for seed yield per plant.

Moderate heritability coupled with moderate genetic advance as per cent of mean was recorded for the trait 100 seed weight. In contrast, low heritability coupled with moderate genetic advance as per cent of mean was reported for number of pods per plant and harvest index. Low heritability coupled with low genetic advance as per cent of mean was reported for number of pods per cluster. These findings were in agreement with Gowsalya *et al* (2016) for 100 seed weight and Gill *et al* (2017) for number of pods per plant

From the foregoing discussion, a perusal of estimates of GCV and PCV delineated all the traits recorded higher PCV compared to GCV. This could be ascribed that a high influence of the environment on the expression of the traits. It suggests that the traits were much affected by environment, and selection on the basis of phenotype independent of genotype could be effective for improvement of such traits.

The difference between GCV and PCV were narrow for days to 50% flowering, days to maturity, number of primary branches per plant, number of seeds per pod and pod length suggesting that these characters are less affected by environmental fluctuations. On the other hand, the characters *viz.*, number of pods per plant, harvest index showed wide differences in the magnitude of GCV and PCV indicating that environmental effect on the expression of these traits is higher. Therefore, the selection based on these characters is not effective for further yield improvement.

Moderate estimates of GCV and higher estimates of PCV were observed for the traits plant height, number of pods per plant, number of clusters per plant and harvest index indicating the presence of ample variation among the genotypes for these traits. Therefore, simple selection could be effective for further improvement of these traits. However, lower estimates of GCV and PCV was observed for the characters days to 50% flowering , days to maturity , number of seeds per pod and pod length indicating the low range of variation for these characters in the genotypes, thus offering little scope for further improvement of these characters through simple selection. Jaberson *et al* (2018) also reported moderate GCV and high PCV for plant height, number of pods per plant and number of clusters per plant. Priya *et al* (2018) revealed low GCV and PCV for days to 50% flowering, number of seeds per pod and pod length.

High estimates of heritability in percentage were observed for days to 50% flowering followed by days to maturity, number of primary branches per plant, number of seeds per pod and pod length. This indicated the influence of additive genetic component for expression of these traits and it demonstrates

that these traits could be successfully transferred to offspring, if selection for these characters is performed in the hybridization programme. These findings were in consonance with Aftab *et al.* (2018) for days to 50% flowering, days to maturity and number of primary branches per plant and khan *et al.* (2020) days to 50% flowering, days to maturity, number of seeds per pod and pod length.

Heritability and genetic advance are indispensable for the parent selection on the basis of quantitative characters. Thus, the traits of genotypes with greater heritability and genetic advance are the foremost demand for the selection of superior genotypes with desirable traits.

In the present investigation, high heritability coupled with high genetic advance as per cent of mean was recorded for number of primary branches per plant specified the dominance of additive gene action in appearance of this trait. Therefore, simple selection might be fruitful for improvement of this character in segregating generation. Priya *et al.* (2018) and Senthamizhselvi *et al.* (2019), Saran *et al.* (2021) and Aman *et al.* (2022) observed high heritability coupled with high genetic advance as per cent of mean number of primary branches per plant. Similarly, high heritability coupled with moderate genetic advance as per cent of mean was recorded for number of seeds per pod suggesting the presence of additive and non additive gene action and the selection may be postponed in succeeding generations. These results were in agreement with Khan *et al.* (2020) for number of seeds per pod.

High heritability coupled with low genetic advance as per cent of mean was recorded for days to 50% flowering, days to maturity and pod length indicating the influence of non additive gene effects (dominance) in the inheritance of these traits and selection for such traits become difficult as the high heritability in these traits is being exhibited due to favourable influence of the environment rather than genotypes. Hence, selection may not be effective for these traits. Similar results were obtained by Aftab *et al.* (2018) for days to 50% flowering and days to maturity; Rehman *et al.* (2020) for pod length and Saran *et al.* (2021) for days to maturity.

Low heritability coupled with low genetic advance as per cent of mean was observed for number of pods per cluster indicating that this trait was governed by non additive gene effects (dominance and/or epistasis) and highly influenced by environmental effects or a combination of these two variables. Hence direct selection for such trait would be ineffective.

From the foregoing discussion, it could be concluded that moderate GCV estimates and high to moderate heritability coupled with high genetic advance as per cent of mean were observed for number of primary branches per plant and number of clusters per plant indicating that the variation in the above characters was most likely due to additive gene effects. Thus, direct selection for these characters may be effective in segregating generations for improvement of seed yield in blackgram.

4.4 GENETIC DIVERGENCE

In any crop improvement programme, assessment of genetic diversity is prerequisite for identifying potential parents for hybridization. Mahalanobis D^2 statistics (Murthy and Arunachalam, 1966) has been found to be a powerful tool in quantifying the degree of genetic divergence among parents, which ensures high heterotic effects and more variability in the segregating generations. It also provides a quantitative measure of association between geographic and genetic diversity based on general distances (Mahalanobis, 1936). The data collected on 12 characters in 35 blackgram genotypes were subjected to Mahalanobis D^2 statistics and the results are presented below.

4.4.1 Mahalanobis D^2 Analysis

To estimate D^2 values, correlated mean of twelve characters of 35 genotypes were transformed into standardized uncorrelated characters using pivotal condensation method (Y1 – Y2). It measures the degree of diversification and determines the relative contribution of each component character to total diversification. The statistical differences (D^2) between pairs of genotypes were obtained as the sum of squares of the differences between the pairs of corresponding uncorrelated values of any two genotypes

considered at a time. Thus, D^2 values were obtained for all the possible [35 (35-1)/2)] 595 pairs of combinations.

4.4.2 Clusters Composition

The 35 genotypes were grouped into eight distinct non over lapping clusters using Tocher's method (Rao, 1952) and distribution of genotypes into each of eight clusters was presented in Table 4.4 and illustrated in Fig 4.1. The distribution of 35 genotypes into eight clusters was at random. Cluster I was the largest with nineteen genotypes followed by cluster III with nine genotypes and cluster V with two genotypes. While the clusters II, IV, VI, VII and VIII were solitary clusters consisting of one genotype each. Panigrahi *et al.* (2014); Patel *et al.* (2014); Gopinath *et al.* (2018); Jayashree *et al.* (2019); Panwar *et al.* (2019); Patel *et al.* (2020) Mallikarjuna *et al.* (2021); Ayesha *et al.* (2021); Chippy *et al.* (2021) and Reni *et al.* (2022) reported grouping of genotypes in different clusters irrespective of their region.

4.4.3 Intra and Inter Cluster Average Distance

The average intra and inter cluster distances (D^2) were estimated according to the procedure given by Singh and Choudhary (1977) and were presented in the Table 4.5 and Fig. 4.2. Divergence analysis revealed that inter cluster distances were greater than intra cluster distances indicating the existence of substantial diversity among the genotypes. Similar results were reported by Panwar *et al.* (2019), Rajalakshmi *et al.* (2020) and Mallikarjuna *et al.* (2021) .

The intra and inter cluster distance among eight clusters were presented in Table 4.5. The intra cluster average distance ranged from 0.00 to 16.42. Among the clusters, cluster III had the maximum intra cluster distance (16.42) followed by cluster I (11.76) and cluster V (6.14), while the clusters II, IV, VI, VII and VIII had recorded zero values as they included only single genotype in each cluster.

Table 4.4. Cluster composition of 35 blackgram genotypes based on Tocher's method

S.No.	Cluster number	Number of genotypes	Genotypes
1	I	19	P-726, UG-708, SB-40-5, RVSU-60, PU-205, SB-25-19, VBG-11-6, TBG-104, VBN-7, COBG-653, PU-31, TU-94-2, P-728, LBG-752, VBG-10-010, KDRS-136, LBG-20, VBG-11-31, WBG-26
2	II	1	VBN-4
3	III	9	LBG-709, MBG-1050, MBG-1051, MBG-1061, NDU-11-204, LBG-22, TU-67, LBG-787, MBG-223
4	IV	1	P-112
5	V	2	LBG-648, LBG-645
6	VI	1	MBG-1058
7	VII	1	P-1032
8	VIII	1	IPU-10-4

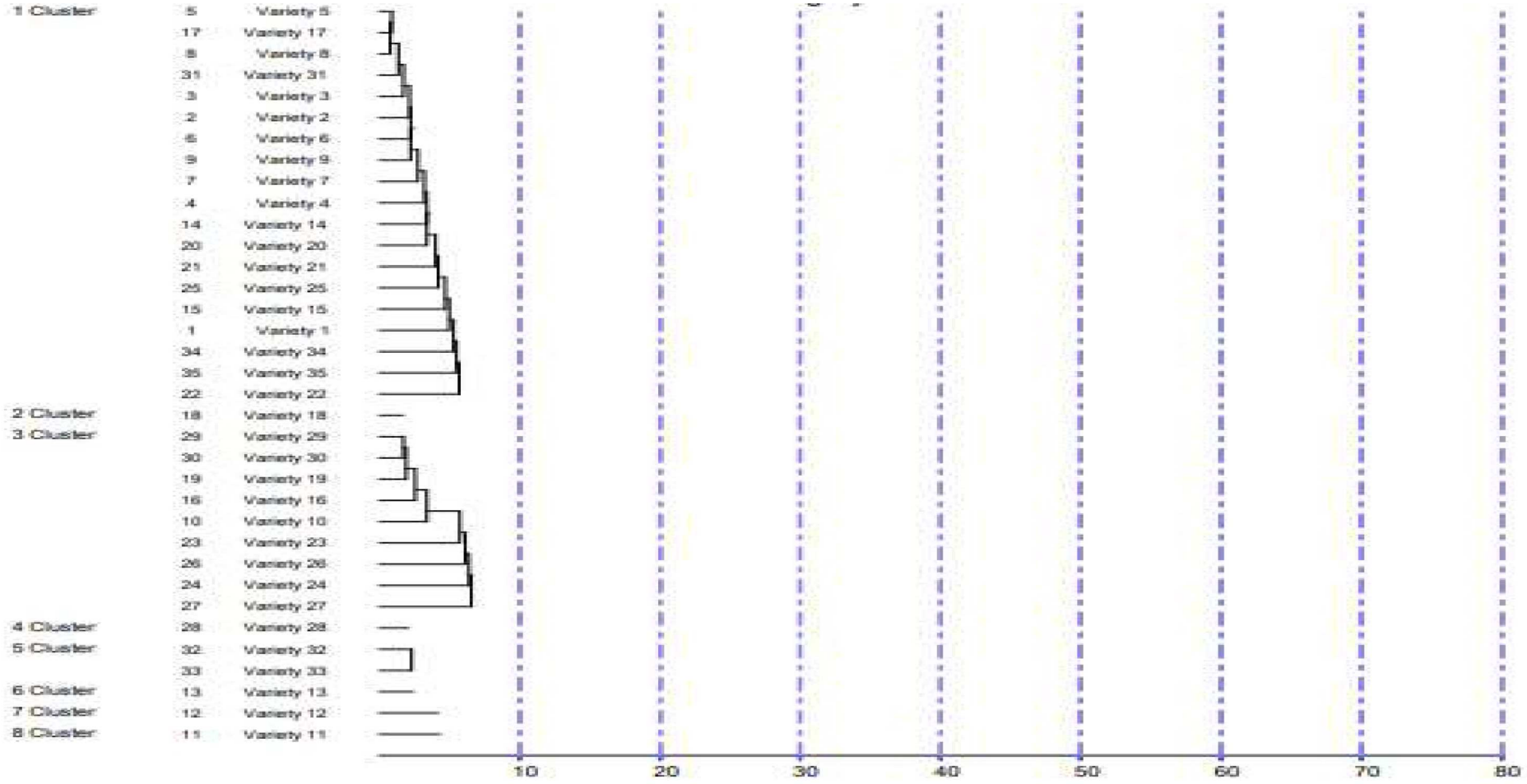


Fig. 4.1. Grouping of 35 blackgram genotypes into 8 clusters using Tocher's method

1.KDRS 136, **2.** SB-25-19, **3.** PU 205, **4.** COBG 653, **5.** P 726, **6.** VBG-11-6, **7.** VBN 7, **8.** SB-40-5, **9.** TBG 104, **10.** NDU-11-204, **11.** IPU-10-4, **12.** P 1032, **13.** MBG 1058, **14.** PU 31, **15.** VBG-10-010, **16.** MBG 1061, **17.** UG 708, **18.** VBN 4, **19.** MBG 1051, **20.** TU-94-2, **21.** P 728, **22.** WBG 26, **23.** LBG 22, **24.** LBG 787, **25.** LBG 752, **26.** TU 67, **27.** MBG 223, **28.** P 112, **29.** LBG 709, **30.** MBG 1050, **31.** RVSU 60, **32.** LBG 648, **33.** LBG 645, **34.** LBG 20, **35.** VBG-11-31.

Table 4.5. Intra cluster (diagonal) and inter cluster distances of eight clusters in 35 blackgram genotypes

Clusters	I	II	III	IV	V	VI	VII	VIII
I	**11.76 (3.43)**	17.97 (4.24)	23.32 (4.83)	18.73 (4.33)	71.27 (8.44)	17.50 (4.18)	21.35 (4.62)	28.04 (5.30)
II		**0.00 (0.00)**	11.77 (3.43)	36.69 (6.06)	52.06 (7.22)	22.95 (4.79)	20.73 (4.55)	31.85 (5.64)
III			**16.42 (4.05)**	44.27 (6.65)	45.01 (6.71)	25.65 (5.06)	28.60 (5.35)	34.62 (5.88)
IV				**0.00 (0.00)**	115.95 (10.77)	39.99 (6.32)	34.22 (5.85)	17.09 (4.13)
V					**6.14 (2.48)**	47.71 (6.91)	57.51 (7.58)	98.16 (9.91)
VI						**0.00 (0.00)**	18.76 (4.33)	52.07 (7.22)
VII							**0.00 (0.00)**	52.74 (7.26)
VIII								**0.00 (0.00)**

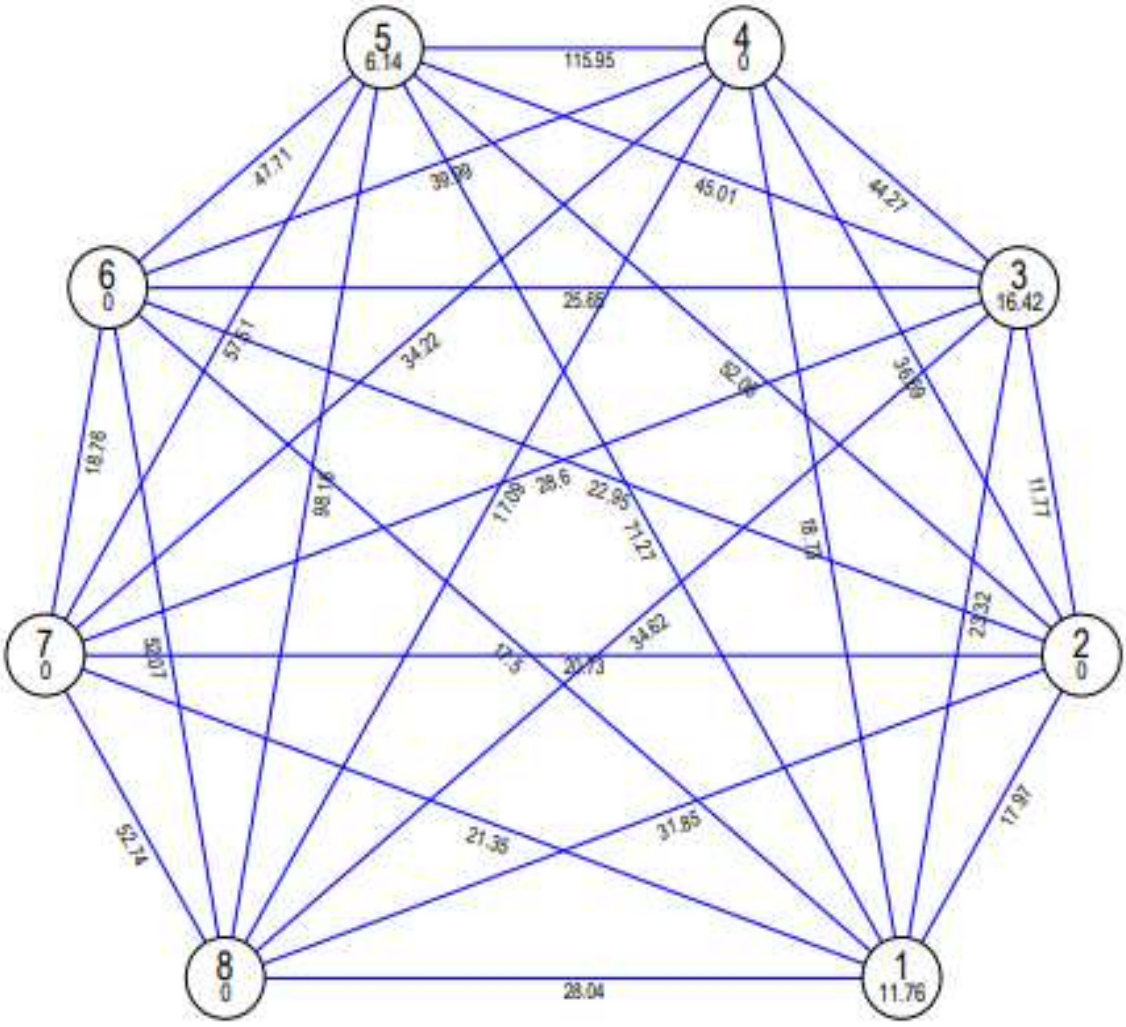


Fig. 4.2. Average intra and inter cluster distances among 35 blackgram genotypes

The maximum inter cluster distance was recorded between cluster IV and V (115.95) followed by cluster V and VIII (98.16), cluster I and V (71.27), cluster V and VII (57.51) and cluster VII and VIII (52.74) indicating that the genotypes belonging to these clusters were genetically more divergent in that order. Conversely, the minimum inter cluster distance was found between cluster II and cluster III (11.77) suggested that the genetic constitution of genotypes in cluster II was in close proximity with the genotypes in the cluster III of that pair.

4.4.4 Cluster Means for Various Characters

The cluster means for each of twelve characters are presented in Table 4.6. Considerable differences between clusters means were observed for most of the characters studied.

Among eight clusters, Cluster IV was early to flower (34.33 days), while cluster II was late to flower (39.00 days). Clusters I, IV, VI and VIII had early flowering genotypes compared to general cluster mean of 37.49 days.

The genotypes in cluster IV had early maturing genotypes (68.33 days) , while the genotypes of cluster V had late maturing genotypes (79.17 days). Clusters I, II, IV, VI and VII had early maturing genotypes compared to general cluster mean of 71.96 days.

The plant height was maximum in cluster V (41.51 cm) and minimum in cluster VIII (17.97 cm). Higher values than the general mean (24.00 cm) was observed in the clusters III, V and VI.

Similarly, number of primary branches per plant varied from 1.73 (cluster VIII) to 4.60 (cluster VI) with a general mean of 3.58. Clusters I, II, V, VI and VII had recorded higher values for number of primary branches per plant than the general mean of 3.58.

Table 4.6. Cluster means with respect to yield and yield attributing traits in 35 blackgram genotypes

Clusters	Characters											
	DFF	**DM**	**PH**	**NPBP**	**NCP**	**NPC**	**NPP**	**NSP**	**PL**	**100 SW**	**SY**	**HI**
I	35.89	70.30	22.36	3.65	6.01	4.16	19.03	6.06	4.62	5.27	4.76	40.02
II	39.00	71.67	20.77	3.80	4.27	4.00	12.87	5.73	4.45	5.63	3.67	32.43
III	38.37	72.85	24.24	3.42	5.30	4.18	17.41	6.15	4.86	5.73	4.26	35.29
IV	34.33	68.33	20.30	3.07	5.67	4.40	21.80	5.33	4.44	4.31	4.90	42.80
V	41.00	79.17	41.51	4.03	7.30	3.37	19.80	6.87	5.21	5.12	5.73	30.17
VI	37.00	73.00	24.13	4.60	7.67	4.80	28.33	6.60	4.91	5.16	5.44	36.59
VII	38.00	68.67	20.73	4.33	5.80	4.00	19.87	5.87	4.98	5.42	6.14	35.66
VIII	36.33	71.67	17.97	1.73	6.03	4.73	25.67	5.60	4.25	5.15	5.19	42.97
Mean	**37.49**	**71.96**	**24.00**	**3.58**	**6.01**	**4.21**	**20.60**	**6.03**	**4.72**	**5.22**	**5.01**	**36.99**

DFF: Days to 50 % flowering, **DM:** Days to maturity, **PH:** Plant height (cm), **NPBP:** Number of primary branches per plant, **NCP:** Number of clusters per plant, **NPC:** Number of pods per cluster, **NPP:** Number of pods per plant, **PL:** Pod length (cm), N**SP:** Number of seeds per pod, **100 SW:**100 seed weight (g), **SYP:** Seed yield per plant (g) and **HI:** Harvest index (%)

Highest number of clusters per plant were registered in cluster VI (7.67) and lowest in cluster II (4.27). Three clusters *viz.,* V, VI, and VIII showed higher values than the general mean of (6.01).

Number of pods per cluster was maximum in cluster VI (4.80) and minimum in cluster V (3.37) with a general mean of 4.21. Clusters IV, VI, and VIII had surpassed the general mean of 4.21.

Cluster means for number of pods per plant differed from 12.87 in cluster II to 28.33 in cluster VI with a general mean of 20.60. Clusters IV, VI, and VIII had recorded higher values for number of pods per plant than the general mean of 20.60.

Cluster IV (5.33) recorded lowest number of seeds per pod, whereas cluster V (6.87) recorded highest number of seeds per pod. Clusters I, III, V and VI had recorded higher values for number of seeds per pod than the general mean of 6.03.

Pod length ranged from 4.25 cm in cluster VIII to 5.21 cm in cluster V. Clusters III, V, VI and VII recorded higher values for pod length than the general mean of 4.72 cm.

The genotypes of cluster III recorded maximum 100 seed weight (5.63 g) while cluster IV recorded minimum (4.31 g) with a general mean of 5.22. Higher values than the general mean (5.22) was observed in the clusters I, II, III and VII.

The genotypes in cluster VII registered high seed yield per plant (6.41 g) while genotypes in cluster II recorded low seed yield per plant (3.67 g). Clusters V, VI, VII, and VIII recorded higher values for seed yield per plant than the general mean of (5.01)

Harvest index was highest in cluster VIII (42.97%) and lowest in cluster V (30.17%). Three clusters viz., I, IV, and VIII showed higher values than the general mean of (36.99).

4.4.5 Relative Contribution of Each Trait towards Diversity

The utility of D^2 analysis is enhanced by its application to estimate the relative contribution of various traits to genetic divergence. The contribution of each trait towards total genetic diversity is presented in Table 4.7.

Among all the traits studied days to 50% flowering contributed maximum (24.2%) to the diversity by ranking 144 times first out of 595 combinations, followed by days to maturity (15.8%) ranking 94 times first, number of seeds per pod (14.12%) ranking 84 times first, number of primary branches per plant (13.78%) ranking 82 times first and pod length (13.11%) ranking 78 times first. The traits *viz.,* seed yield per plant, harvest index, number of clusters per plant, 100 seed weight, plant height, number of pods per cluster and number of pods per plant contributed 7.56%, 4.20%, 3.87%, 2.18%, 0.84%, 0.17% and 0.17%, respectively to the genetic divergence in decreasing order. These results were in consonance with the findings of Gopinath *et al.* (2018) for pod length and harvest index; Senthilkumar (2018) for 100 seed weight; Jayashree *et al.* (2019) for days to 50 per cent flowering; Pratap *et al.* (2020); for days to maturity; Rajalakshmi *et al.* (2020) for seed yield per plant; Chippy *et al.* (2021) for 100 seed weight; Mallikarjuna *et al. q1* 2021 for pod length and Goswami *et al.* (2022) for number of primary branches per plant and pod length;

Information on genetic diversity and quantitative traits usually help the breeder for choosing superior characters as well as good parents. Thus, the results of the genetic divergence study of the genotypes of this experiment will be helpful to select superior genotypes for development of high yielding blackgram varieties.

Table 4.7. Relative contribution of yield and yield attributing traits towards genetic divergence in 35 blackgram genotypes

S. No	Characters	Number of times ranked first	Contribution (%)
1	Days to 50 % flowering	144	24.2%
2	Days to maturity	94	15.8%
3	Plant height (cm)	5	0.84%
4	Number of primary branches per plant	82	13.78%
5	Number of clusters per plant	23	3.87%
6	Number of pods per cluster	1	0.17%
7	Number of pods per plant	1	0.17%
8	Number of seeds per pod	84	14.12%
9	Pod length (cm)	78	13.11%
10	100 seed weight (g)	13	2.18%
11	Seed yield per plant (g)	45	7.56%
12	Harvest index (%)	25	4.2%

Based on the results obtained from diversity analysis, all the genotypes were grouped in to eight clusters, of which cluster I contained maximum number of 19 genotypes followed by cluster III with 9 genotypes and cluster V with 2 genotypes, whereas clusters II, IV, VI, VII and VIII comprised of only one genotype each. Grouping of genotypes into clusters delineated the genotypes from different regions were grouped in same cluster and the genotypes from same region were grouped in different clusters. Hence, the clustering of genotypes from different geographical regions together into same cluster implying that there were more similarities than differences between the collected genotypes. The grouping of genotypes from same source into different clusters may be due to the continuous selection by the breeders in similar environmental constraints across regions may have narrowed the phenotypic diversity and also the free exchange of breeding material among different regions could explain the similarity between genotypes

The pattern of grouping genotypes in different cluster proved that geographical distribution and genetic divergence did not follow the same pattern and the existence of significant amount of variability. Therefore, it can be concluded that the selection of parents for hybridization should not be based on geographical diversity only, but it should have a base of both geographical origin as well as genetic divergence. These results were also in confirmity with the findings of Rajalakshmi *et al.* (2020); Mallikarjuna *et al.* (2021); Ayesha *et al* (2021); Chippy *et al.* (2021) and Reni *et al.* (2022).

Inter cluster distances were higher than the intra cluster distances which indicated substantial diversity present among the genotypes studied. The maximum inter cluster distance was observed between cluster IV and V followed by cluster V and VIII, cluster I and V, cluster V and VII and cluster VII and VIII indicating that the genotypes belonging to these clusters were genetically more divergent in that order. The genotypes in these clusters may serve as potential parents and crossing between these genotypes may result in

novel recombinants. In contrast, inter cluster distance was minimum between cluster II and III (11.77) indicating a close relationship and similar magnitude for most of the traits of the genotypes in these clusters.

Cluster means for different characters showed considerable differences between the clusters for all the characters. Cluster VI recorded maximum mean values for number of primary branches per plant, number of clusters per plant, number of pods per cluster and number of pods per plant. Similarly Cluster V registered higher values for plant height, number of seeds per pod and pod length. Cluster IV showed minimum values for days to 50 per cent flowering and days to maturity. Cluster III for 100 seed weight; Cluster VII for seed yield per plant and cluster VIII for harvest index recorded maximum values. Inter crossing the genotypes from these clusters could be suggested to generate wide range of variability subsequently followed by effective selection for these characters. Hence, selection of genotypes from cluster VI, V, III, VII and VIII could be recommended for development of high yielding blackgram varieties. Similarly, genotypes from the cluster IV could be used as parents in hybridization programme for evolving short duration varieties.

The traits *viz.*, days to 50% flowering followed by days to maturity, number of seeds per pod, number of primary branches per plant and pod length contributed relatively maximum towards the total divergence. Therefore, selection for these traits might be given more emphasis in hybridization programme to generate large variability and will provide immense scope for the improvement of yield through selection. These results are in line with Punithavathy *et al.* (2020) for pod length and number of primary branches per plant and Reni *et al.* (2022) for days to maturity.

From the above discussion, It could be concluded that for getting most promising segregants, selection of genotypes should be between cluster possessing maximum genetic divergence (cluster IV and V, cluster V and cluster VIII, cluster V and cluster I, cluster V and cluster VII and cluster VII

and cluster VII) and having superior performance for the desirable traits. Hence, the crosses *viz.,* LBG-645 × P-112 (cluster V × cluster IV), LBG-645 × IP4-10-4 (cluster V × cluster VIII), LBG-645 × PU-31 (cluster V × cluster I), LBG-645 × P-1032 (cluster V × cluster VII) and P-1032 × IPU-10-4 (cluster VII × cluster VIII) could be suggested for yield improvement in blackgram.

4.5 GENOTYPE BY TRAIT BIPLOT

The primary goal of any breeding program is to improve the yield. Yield being an intricate character, which is influenced by a number of inter related traits, the interdependence of these characters will influence yield either directly or indirectly. The nature of association between yield and its components helps in simultaneous selection for many characters associated with yield improvement. Genotype by trait (GT) analysis presents the results of trait relationship by graphical display of the genetic relationships among traits It offers a visual comparison among genotypes on the the basis of multiple traits and it can be helpful in independent culling based on multiple traits and in comparing selection strategies, which are important for both cultivar evaluation and parental selection (Yan and Rajcan, 2002; Yan and Tinker, 2005).

GT biplot technique has been applied in many crops *viz.,* soybeans (Yan and Rajcan 2002), barley (Yan & Tinker, 2005), maize (Apraku *et al.,* 2010), wheat (Mohammadi *et al.,* 2011), cowpea (Oladejo *et al.,* 2011), sugarcane (Chatwachirawong *et al.,* 2012), Groundnut (Safari *et al.,* 2013), greengram (Paramesh, 2014) and foxtail millet (Reddy *et al*., 2015) to identify the relationships between traits and to evaluate the genotypes on the basis of multiple traits.

4.5.1 GT Biplot Analysis of Inter Relationship among Yield and Yield Attributing Traits

A vector view of GT biplot (Fig 4.3) represented the interrelationship among all the traits measured. The GT biplot is constructed by the first two principal components (PC1 and PC2, also referred to as primary and secondary effects, respectively) derived from yield data and its component traits. (Yan, 1999; Yan *et al.,* 2000). Principal components PC1 (36.49%) and PC2 (21.34%) explained 57.83% of the total variation observed among the cultivars based on all the traits.

The lines connecting each trait marker to the origin of the biplot are called the trait vectors and the length of each trait vector approximates the standard deviation of each trait. The cosine of the angle between the vectors of any two traits approximates the correlation coefficient (degree of association) between the traits. On this premise, If there is acute angle (< 90°) between two vectors then they are positively correlated. Whereas, if there is obtuse angle (> 90°) between them then these are negatively associated but in case of right angle (=90°), vectors are said to be independent. (Yan *et al*., 2007).

Among the 35 tested genotypes, seed yield per plant was highly positively correlated (an acute angle) with number of pods per plant, number of clusters per plant and number of primary branches per plant. Similarly, it was also positively correlated with number of seeds per pod, plant height, days to maturity, pod length, number of pods per cluster and harvest index. In contrast, seed yield was negatively correlated (obtuse angle) with days to 50 % flowering and 100 seed weight. However, the magnitude of the negative association of days to 50 % flowering with seed yield per plant was low suggesting that genotypes with early flowering and high seed yield could be selected for improvement of these traits.

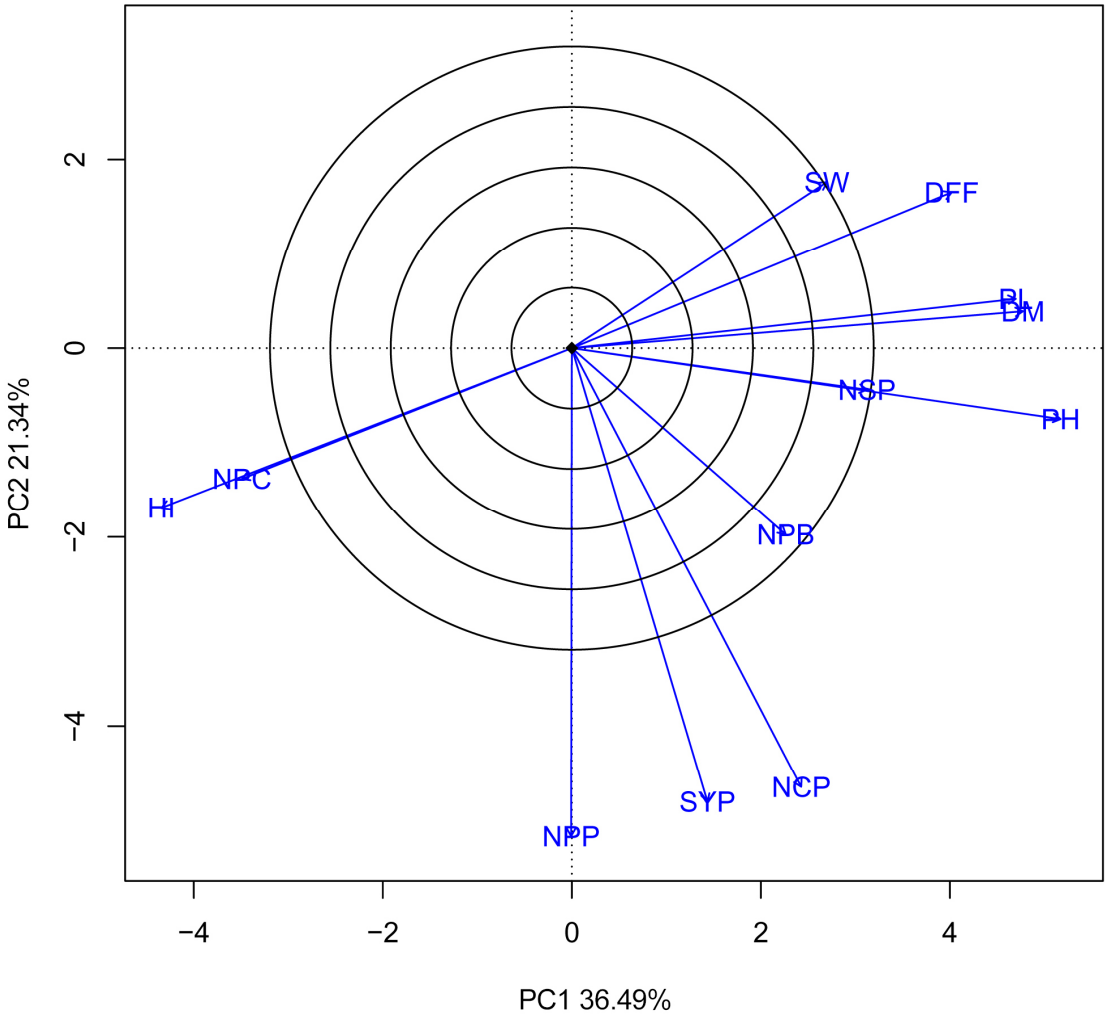


Fig. 4.3. GT Biplot Analysis of inter relationship among yield and yield attributing traits of 35 blackgram genotypes

DFF: Days to 50 % flowering, **DM:** Days to maturity, **PH:** Plant height (cm), **NPBP:** Number of primary branches per plant, **NCP:** Number of clusters per plant, **NPC:** Number of pods per cluster, **NPP:** Number of pods per plant, **PL:** Pod length (cm), N**SP:** Number of seeds per pod, **100 SW:**100 seed weight (g), **SYP:** Seed yield per plant (g) and **HI:** Harvest index (%)

The trait, number of pods per plant was positively correlated with seed yield per plant, number of clusters per plant, number of primary branches per plant, number of seeds per pod, plant height, number of pods per cluster and harvest index. However, it was negatively correlated with days to maturity, pod length, days to 50 % flowering and 100 seed weight. Similarly, number of clusters per plant exhibited positive correlation with number of pods per plant, seed yield per plant, number of primary branches per plant, number of seeds per pod, plant height, days to maturity, pod length and days to 50 % flowering. In contrast, it was negatively correlated with 100 seed weight, number of pods per cluster and harvest index.

Number of primary branches per plant was positively correlated with all the traits except harvest index and number of pods per clusters to which it was negatively correlated. The traits number of seeds per pod and plant height were strongly correlated, both were positively correlated with all the traits except harvest index and number of pods per clusters to which they were negatively correlated. Similarly, the traits days to maturity and pod length are closely correlated, both were positively correlated with seed yield per plant, number of primary branches per plant, number of clusters per plant, number of seeds per pod, plant height, days to 50 % flowering and 100 seed weight and slightly negatively correlated with number of pods per plant. They showed strong negative correlation with number of pods per cluster and harvest index.

The trait days to 50 % flowering showed positive correlation with 100 seed weight, days to maturity, pod length, number of seeds per pod, plant height, number of primary branches per plant and number of clusters per plant and slightly negatively correlated with seed yield per plant. However, this trait exhibited strong negative correlation with number of pods per plant, number of pods per cluster and harvest index. The traits days to 50 % flowering, days to maturity, pod length, number of seeds per pod, plant height and number of primary branches per plant were positively correlated with hundred seed weight, while the traits number of clusters per plant, seed yield per plant,

number of pods per plant, number of pods per cluster and harvest index were negatively correlated. The traits number of pods per cluster and harvest index are strongly correlated, both showed negative correlation with all the traits. Similarly, Oladejo *et al.* (2011) in cowpea, Singh *et al.* (2014) in greengram, Paramesh (2014) in mungbean, Sofia (2016) in greengram Sharma *et al.* (2018) in blackgram and Kavitha *et al.* (2021) in blackgram used GT biplot technique to understand association among yield and yield attributing traits

Based on perusal of trait inter relationships biplot, it could be concluded that the traits number of pods per plant, number of clusters per plant, number of primary branches per plant, number of seeds per pod and plant height are the most reliable traits for indirect selection for improvement of seed yield in blackgram.

4.5.2 Discriminativeness and Representativeness of yield and yield attributing traits

Figure 4.4 of the GT Biplot shows the discriminating ability and representativeness of the traits under study. The biplot accounted for 57.83 % of the variation among the genotypes for the measured traits. The concentric circles on the biplot help to visualize the length of the trait vectors, which is proportional to the standard deviation within the respective traits. It is a measure of the discriminating ability of the traits. Longer vector length showed more discriminating power relative to vectors of shorter length. Among the twelve traits, number of pods per plant, number of clusters per plant and plant height were most discriminating (informative), whereas 100 seed weight was least or non discriminating. Non discriminating traits (those with very short vectors) are less useful as they provide little discriminating information about the genotypes.

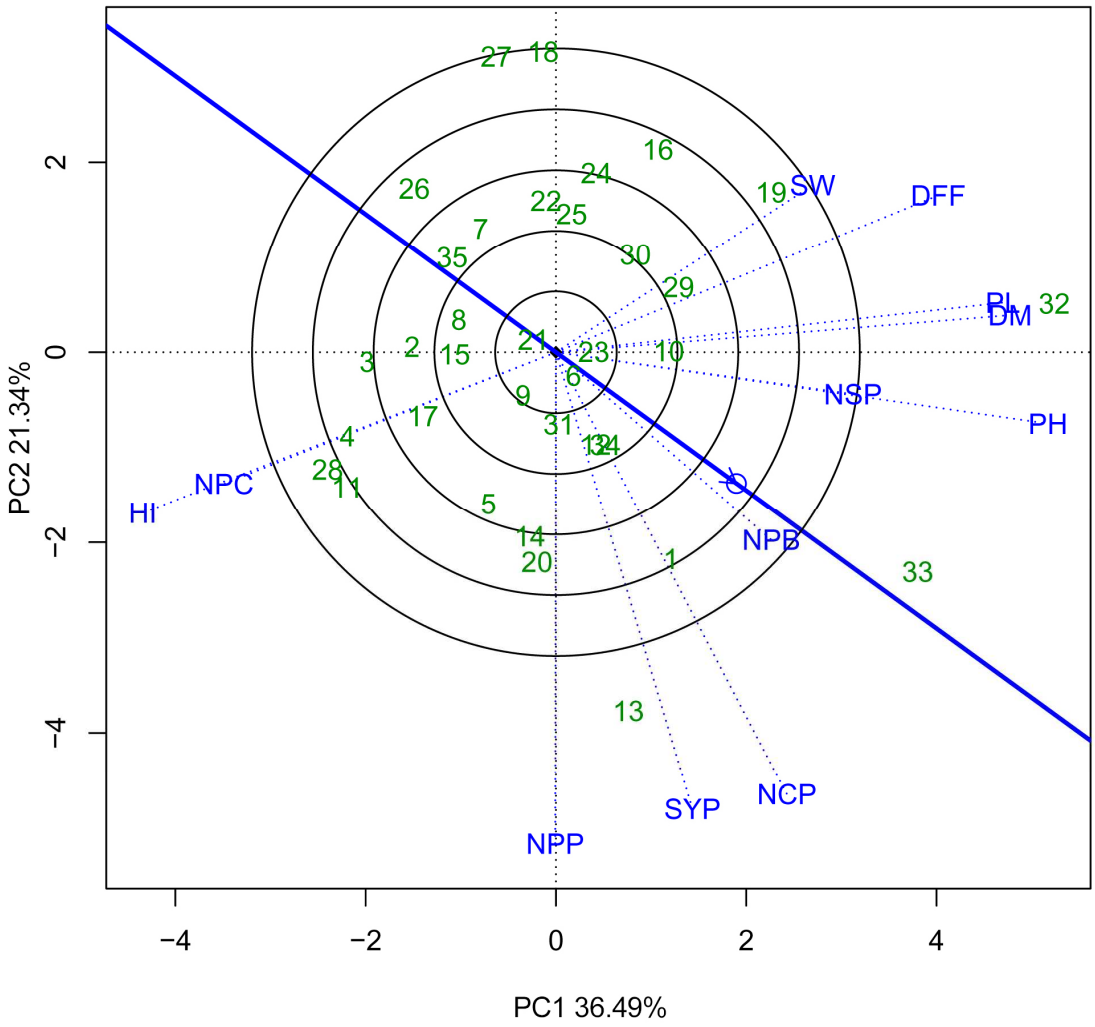


Fig. 4.4. Discriminativeness and representativeness of yield and yield attributing traits of 35 blackgram genotypes

DFF: Days to 50 % flowering, **DM:** Days to maturity, **PH:** Plant height (cm), **NPBP:** Number of primary branches per plant, **NCP:** Number of clusters per plant, **NPC:** Number of pods per cluster, **NPP:** Number of pods per plant, **PL:** Pod length (cm), N**SP:** Number of seeds per pod, **100 SW:**100 seed weight (g), **SYP:** Seed yield per plant (g) and **HI:** Harvest index (%)

Average treatment is displayed by arrow in biplot graph. Vector of average treatment is called average treatment axis or average tester axis (Yan, 2001). Average tester axis (ATA) is the vector line that passes through the average treatment and origin of biplot. A trait that has a smaller angle with the ATA is more representative of other traits. Thus, number of primary branches per plant is most representative followed by number of clusters per plant and plant height, whereas numbers of pods per cluster and harvest index are least representative.

4.5.3 GT Biplot Analysis for Ranking of Yield and Yield Attributing Traits

The vector view of the GT biplot (Fig. 4.5) helps to rank traits relative to an ideal trait (represented by center of the concentric circles). Among the traits studied, the ideal trait should be most discriminating (informative) and also most representative of the target trait. An “ideal trait” is the one which is present in the center of the concentric circles. It is a point on the ATA in the positive direction (“most representative”) with a distance to the biplot origin equal to the longest vector of all traits (“most informative”). The biplot accounted for 57.83 % of the total variation among the varieties for the measured traits, which was partitioned as 36.49 % explained by the first principal component (PC1) and 21.34 % explained by the second principal component (PC2).

Based on their discriminating ability and representativeness, the traits may be ranked as follows; Number of primary branches per plant ≈ number of clusters per plant ≈ plant height > number of seeds per pod > see yield per plant ≈ days to maturity ≈ pod length > days to 50 % flowering > number of pods per plant > 100 seed weight > number of pods per cluster > harvest index.

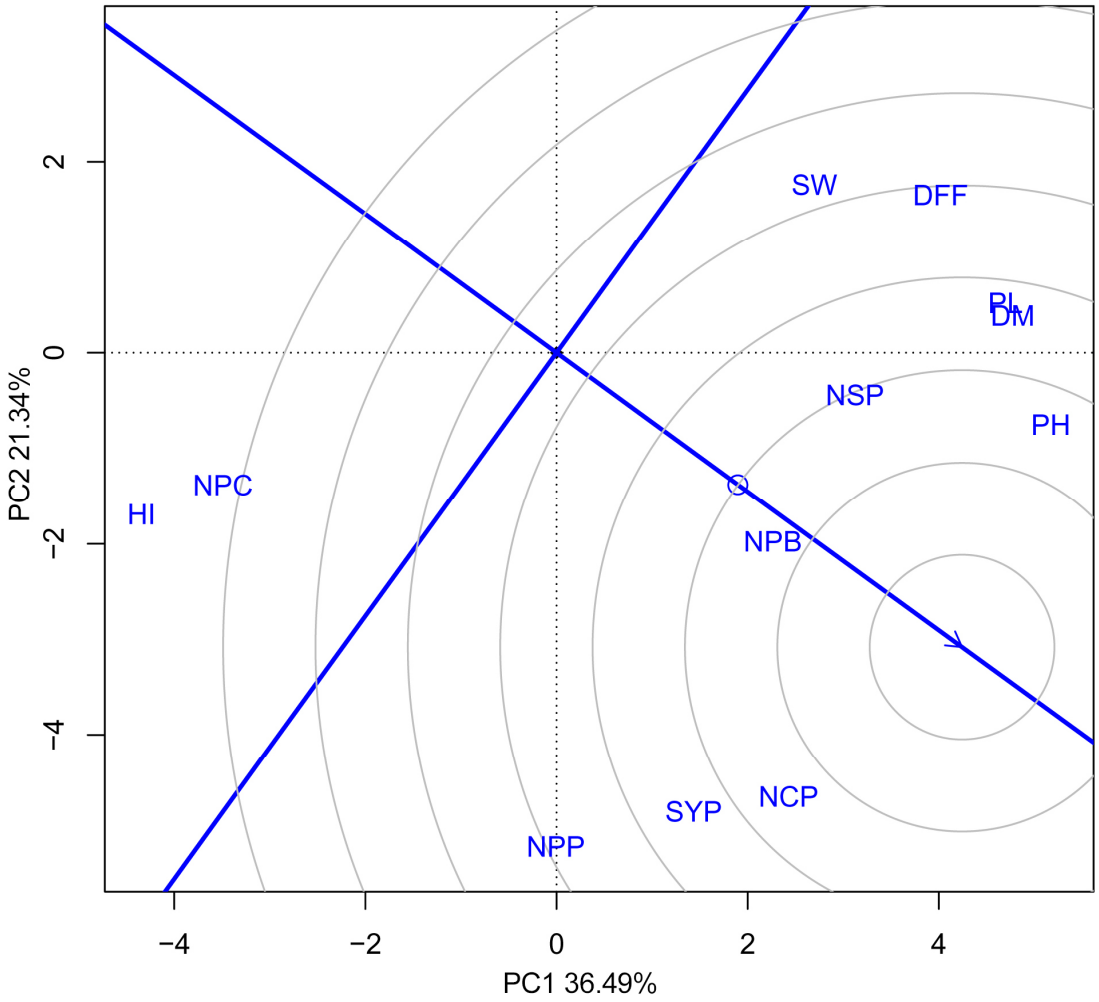


Fig. 4.5. GT biplot analysis for ranking of yield and yield attributing traits of 35 blackgram genotypes

DFF: Days to 50 % flowering, **DM:** Days to maturity, **PH:** Plant height (cm), **NPBP:** Number of primary branches per plant, **NCP:** Number of clusters per plant, **NPC:** Number of pods per cluster, **NPP:** Number of pods per plant, **PL:** Pod length (cm), N**SP:** Number of seeds per pod, **100 SW:**100 seed weight (g), **SYP:** Seed yield per plant (g) and **HI:** Harvest index (%)

The traits number of primary branches per plant, number of clusters per plant and plant height were identified as ideal traits followed by number of seeds per pod. Therefore, these traits should be considered simultaneously as effective selection criteria evolving high yielding blackgram genotypes.

4.5.4 GT Biplot Analysis for Ranking of Genotypes

A vector view of genotype by trait biplot representing the ranking of 35 blackgram genotypes is presented in Fig 4.6. Principal components for PC1 and PC2 explained 57.83% of the variation among the traits. The biplot was based on trait focused singular value partitioning (SVP = 1) and therefore, appropriate for visualizing the relationships among genotypes. The horizontal line passing through the biplot origin and the average tester, with an arrow pointing to the average tester and its ordinate passing through the origin and perpendicular to the abscissa is called the average tester axis or ATC abscissa.

An ideal genotype is a virtual genotype and its position procedure of GT biplot analysis would be most appropriate application for selection of high yielding genotypes. A genotype was said to be ideal if it was present at the center of concentric circles and in the positive direction of ATA in GT biplot. Therefore, genotypes located closer to the 'ideal genotype' are more desirable than others.

Based on the relative position of the genotypes from ideal cultivar they may be ranked as follows; LBG-645 > KDRS-136 > MBG-1058 > LBG-648 ≈ P-1032 ≈ LBG-20 ≈ NDU-11-204 > PU-31 ≈ TU-94-2 > RVSU-60 ≈ LBG-709 > VBG-11-6 ≈ LBG-22 > P 726 ≈ TBG-104 ≈ MBG-1051 ≈ MBG-1050 > P-728 > UG-708 ≈ VBG-10-010 ≈ LBG-752 ≈ MBG-1061 > SB-40-5 ≈ WBG-26 ≈ LBG-787 > IPU-10-4 ≈ SB-25-19 ≈ VBG-11-31 ≈ VBN-7 > P-112 ≈ COBG-653 ≈ PU-205 ≈ TU-67 > VBN-4 > MBG-223.

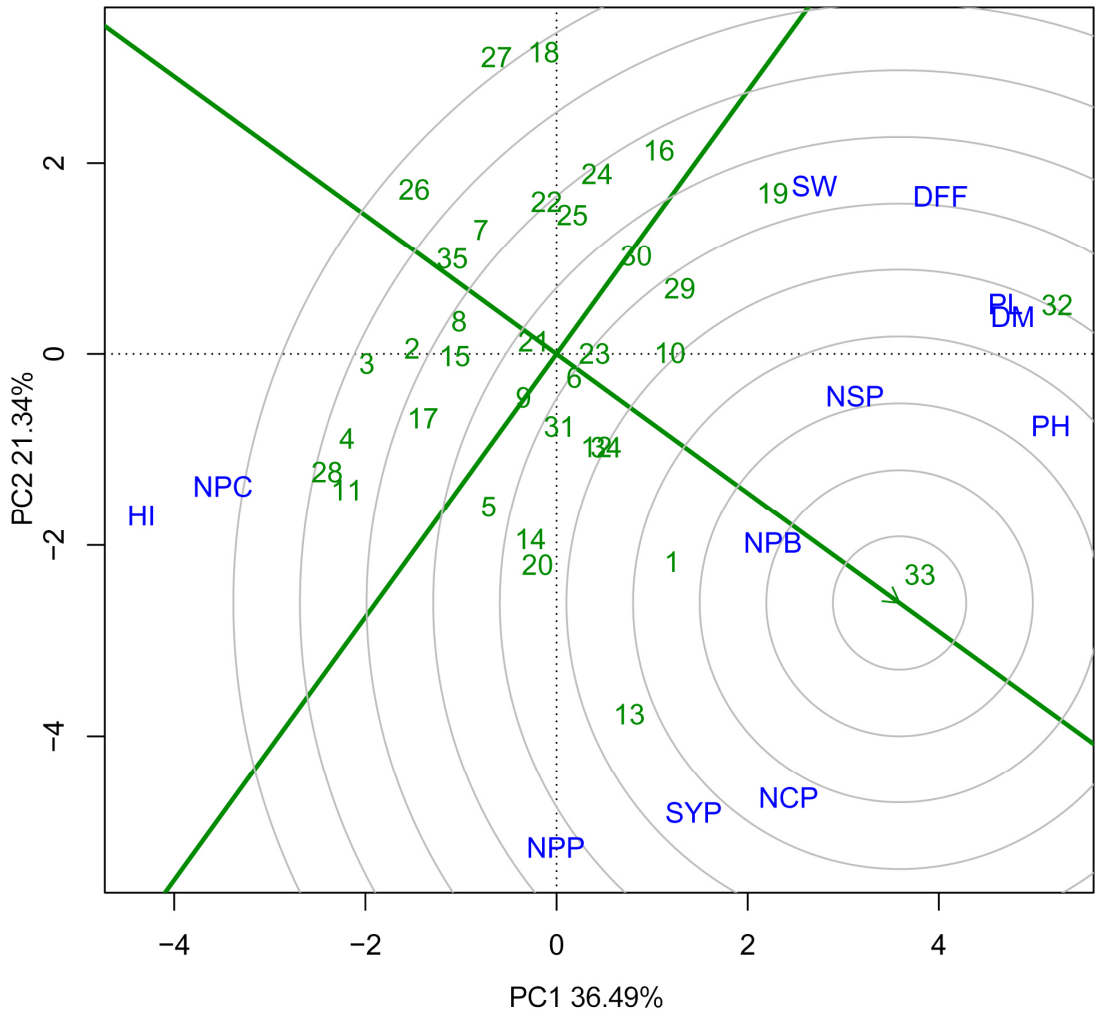


Fig. 4.6. GT biplot analysis for ranking of 35 blackgram genotypes

1.KDRS 136, **2.** SB-25-19, **3.** PU 205, **4.** COBG 653, **5.** P 726, **6.** VBG-11-6, **7.** VBN 7, **8.** SB-40-5, **9.** TBG 104, **10.** NDU-11-204, **11.** IPU-10-4, **12.** P 1032, **13.** MBG 1058, **14.** PU 31, **15.** VBG-10-010, **16.** MBG 1061, **17.** UG 708, **18.** VBN 4, **19.** MBG 1051, **20.** TU-94-2, **21.** P 728, **22.** WBG 26, **23.** LBG 22, **24.** LBG 787, **25.** LBG 752, **26.** TU 67, **27.** MBG 223, **28.** P 112, **29.** LBG 709, **30.** MBG 1050, **31.** RVSU 60, **32.** LBG 648, **33.** LBG 645, **34.** LBG 20, **35.** VBG-11-31.

The genotype LBG-645 was identified as the ideal cultivar followed by KDRS-136, MBG-1058, LBG-648, P-1032, LBG-20 and NDU-11-204. Similarly, several workers *viz.,* Yan and Rajcan (2002) in soybean, Hamid *et al.* (2008) in rapeseed, Safari *et al*. (2013) in groundnut, Apraku *et al*. (2010) in maize and Paramesh (2014) in greengram and Kavitha *et al.* (2021) in blackgram used GT biplot technique in ranking and identification of the best genotypes.

4.5.5 Genotype by Trait Interaction Biplot for Yield and Yield Attributing Traits in 35 Blackgram genotypes

Study of genotype and trait interaction is very important for evaluation of genotypes. The biplot showed relationship between traits by angle formed between two or more variables for 35 blackgram genotypes (Fig 4.7). It can also be used to compare genotypes on the basis of multiple traits and to identify genotypes that are particularly good in certain aspects. The biplot accounted for a substantial proportion (57.83 %) of the variation among genotypes for traits studied.

From Fig 4.7, it was represented that the genotypes LBG-645, MBG-1058, KDRS-136, P-1032, LBG-20 and VBG-11-6 exhibited superior performance for seed yield per plant, number of clusters per plant, number of primary branches per plant indicating that these genotypes could be used as parents in the breeding programs aimed towards development of high yielding genotypes in blackgram. The genotypes LBG-22, NDU-11-204, LBG-648 showed better performance for number of seeds per pod, plant height, pod length and days to maturity. Similarly, the genotypes TU-94-2, PU-31 and RVSU-60 for number of pods per plant; the genotypes MBG-1051, MBG-1050 and LBG-709 for 100 seed weight; the genotypes COBG-653 and UG-708 for harvest index and number of pods per cluster exhibited superior performance for respective traits. Therefore, these genotypes could be utilized for the improvement of the respective traits in blackgram.

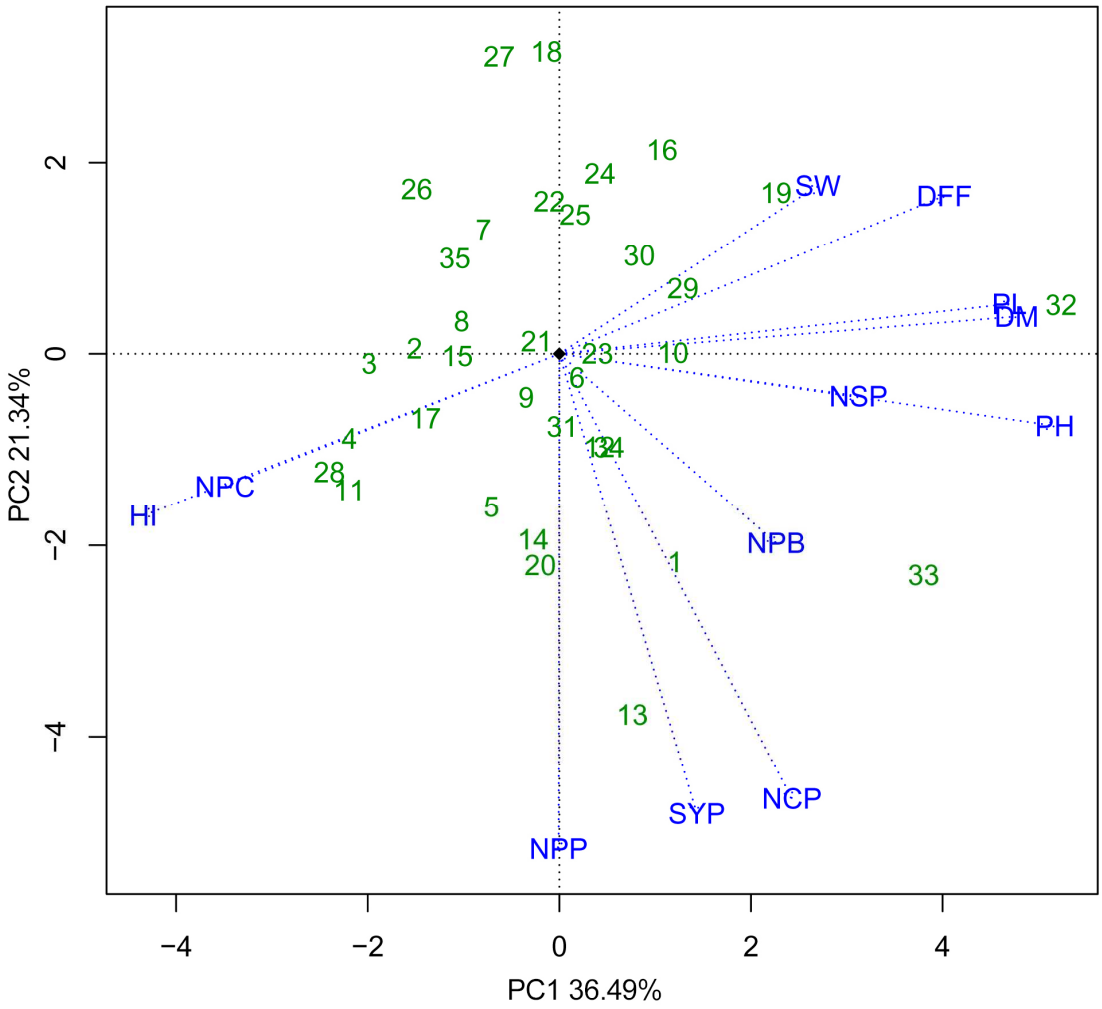


Fig. 4.7. Genotype by trait interaction biplot for yield and yield attributing traits in 35 blackgram genotypes

1.KDRS 136, **2.** SB-25-19, **3.** PU 205, **4.** COBG 653, **5.** P 726, **6.** VBG-11-6, **7.** VBN 7, **8.** SB-40-5, **9.** TBG 104, **10.** NDU-11-204, **11.** IPU-10-4, **12.** P 1032, **13.** MBG 1058, **14.** PU 31, **15.** VBG-10-010, **16.** MBG 1061, **17.** UG 708, **18.** VBN 4, **19.** MBG 1051, **20.** TU-94-2, **21.** P 728, **22.** WBG 26, **23.** LBG 22, **24.** LBG 787, **25.** LBG 752, **26.** TU 67, **27.** MBG 223, **28.** P 112, **29.** LBG 709, **30.** MBG 1050, **31.** RVSU 60, **32.** LBG 648, **33.** LBG 645, **34.** LBG 20, **35.** VBG-11-31.

DFF: Days to 50 % flowering, **DM:** Days to maturity, **PH:** Plant height (cm), **NPBP:** Number of primary branches per plant, **NCP:** Number of clusters per plant, **NPC:** Number of pods per cluster, **NPP:** Number of pods per plant, **PL:** Pod length (cm), N**SP:** Number of seeds per pod, **100 SW:**100 seed weight (g), **SYP:** Seed yield per plant (g) and **HI:** Harvest index (%)

The GT biplots showed rich information that can be utilized by breeders, especially in selection of parents for a breeding programme aimed at improving particular traits, as was pointed by Yan and Kang (2003), and Yan and Rajcan (2002). However, an exact match with mean performance is not expected, because the biplot describes the interrelationships among all traits on the basis of overall pattern of the data (Yan and Reid, 2008).

From the foregoing discussion on trait association using GT biplot analysis for yield and yield attributing traits, among 35 blackgram genotypes seed yield per plant was highly positively correlated with number of pods per plant, number of clusters per plant and number of primary branches per plant and also positively correlated with number of seeds per pod, plant height, days to maturity, pod length, number of pods per cluster and harvest index, indicating that an increase in the magnitude of any of these traits will lead to subsequent increase in the magnitude of seed yield. Hence, these traits could be used in the further selection programme for improvement of seed yield per plant. In contrast, seed yield per plant was negatively correlated with 100 seed weight. Hence, recurrent selection procedures with intensive intermatings could be suggested to improve high seed yield coupled with bold seededness in blackgram.

By and large, based on GT biplot, it could be concluded that, the genotypes *viz.*, LBG-645, KDRS-136, MBG-1058, LBG-648, P-1032, LBG-20 and NDU-11-204 were identified as ideal cultivars. Therefore, selection of these genotypes as a potential donors in future breeding programmes would be more rewarding for improving seed yield in blackgram. Similarly, the traits number of primary branches per plant, number of clusters per plant and plant height were found to be the ideal traits with high discriminativeness and representativeness. Hence, these traits could be selected aiming for improvement of seed yield in blackgram.

4.6. MOLECULAR PROFILING

The constant and ever increasing number of improved varieties leads to narrow genetic base and creates an interruption in varietal identification. Unambiguous identification of varieties is exceedingly important for registration and certification of newly released and notified varieties. Molecular profiling is an effective method to identify genotypes using molecular markers. As PCR based molecular markers especially SSR's are very quick, reliable, environmentally neutral in varietal profiling and for accommodating in the development of unambiguous DNA profiles of cultivars, the present investigation is carried out using sixteen SSR markers. Similar technique was used by many scientists to differentiate varieties in various crops *viz.,* Chakravarthi and Naravaneni, (2006) in rice, Agarwal and Katiyar, (2008) in lentil, Siew *et al.* (2018) in durian, Amaravathi *et al.* (2014) in groundnut, Barghavi *et al.* (2021) in rice and Panigrahi *et al.* (2020) in blackgram.

Out of sixteen SSR markers, six were monomorphic and the remaining ten markers generated 34 polymorphic loci. Out of ten polymorphic markers six markers *viz.,* CEDG 006, CEDG 139, CEDG 20, CED 173, CEDG 180 and CEDG 44 were distributed on linkage groups (LG) 2, 4, 5, 9, 10, 11 respectively, whereas the linkage groups of markers BM 170, CEDG 198, CEDG 225, CEDG 243 were unambiguous. Representative gel profiles of 35 blackgram genotypes with SSR markers CEDG 44 and BM 170 were shown in Plate. 2.

4.6.1 Number and Frequency of Alleles

The polymorphic primers generated alleles ranging from 2 to 6 with an average of 3.4 per locus. Highest number of six alleles were detected with CEDG 20, while it was lowest of two alleles with CEDG 173 and CEDG 225. The frequency of the alleles ranged from 0.03 to 0.89. A 113bp allele amplified by the marker CEDG 006 showed highest frequency, being present in 31 cultivars out of 35 tested. The alleles 124bp, 207bp, and 290bp produced by the markers CEDG 006, CEDG 20 and BM 170 respectively showed least frequency, being amplified in one cultivar each *viz.,* LBG-752, KDRS-136 and PU-205 respectively.

4.6.2 Unique Alleles

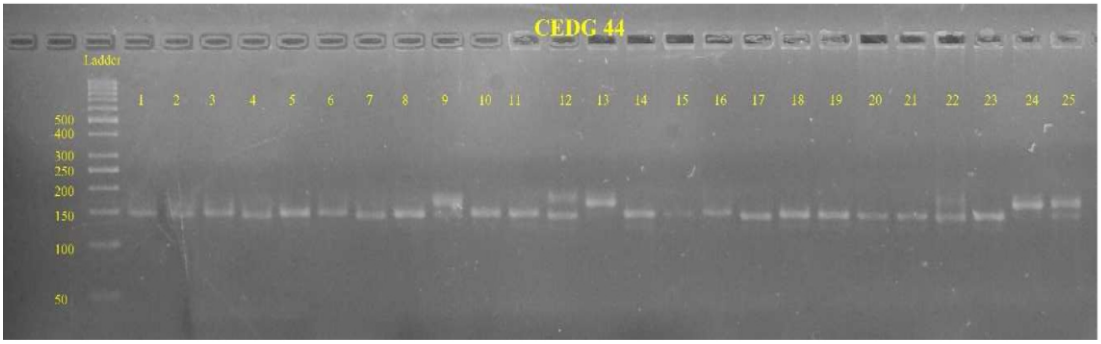
The three unique alleles that were found with markers CEDG 006 for LBG-752 (124bp), CEDG 20 for KDRS-136 (207bp), BM 170 for PU-205 (290bp) can be considered as cultivar specific alleles. Hence, these alleles could be used for identification of these varieties directly. Sequencing of these alleles furthers reveals additional molecular depths if any of the cultivars.

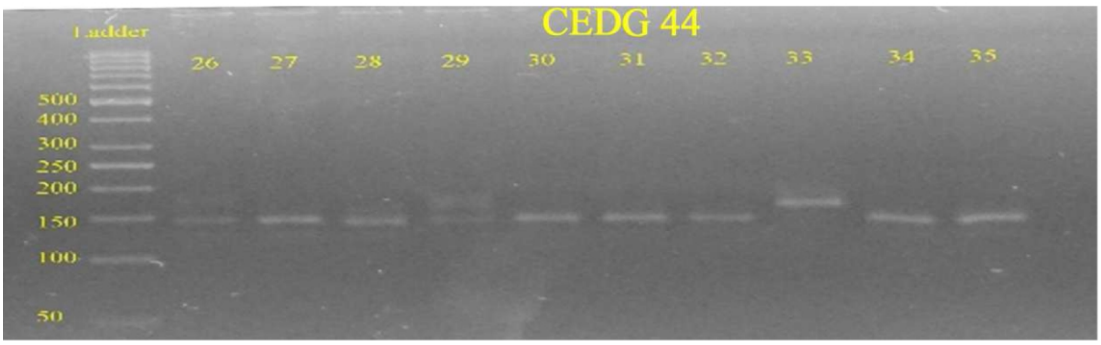
4.6.3 Polymorphism Information Content of SSR Markers

Polymorphism information content (PIC) provides an estimate of the discriminatory power of the marker taking into account both the number and relative frequency of the alleles and the values ranged from 0 (monomorphic) to 1 (Highly polymorphic). In this study, PIC values in polymorphic markers ranged from 0.21 (CEDG 006) to 0.79 (CEDG 20) with 0.57 as average (Table 4.8). PIC values in the present study were lower than the previous reports of Baisakh *et al.* 2021 (0.75). This might be due to limited number of genotypes screened and varietal difference. The polymorphic markers with high PIC (>0.5) have been regarded as highly informative markers. Out of 10 polymorphic SSR markers, 8 of them displayed PIC values above 0.5 (Table 4.8). PIC information along with number of alleles provides reliable information on highly informative SSR markers for universal fingerprinting and estimation of genetic diversity. Based on the level of polymorphism detected by individual marker, four most informative SSR markers namely, CEDG 20 (0.792), CEDG 44 (0.656), CEDG 243 (0.647) and BM 170 (0.629) were identified which displayed very high PIC values. This set of markers had the enormous potential to identify most of the blackgram genotypes under study.

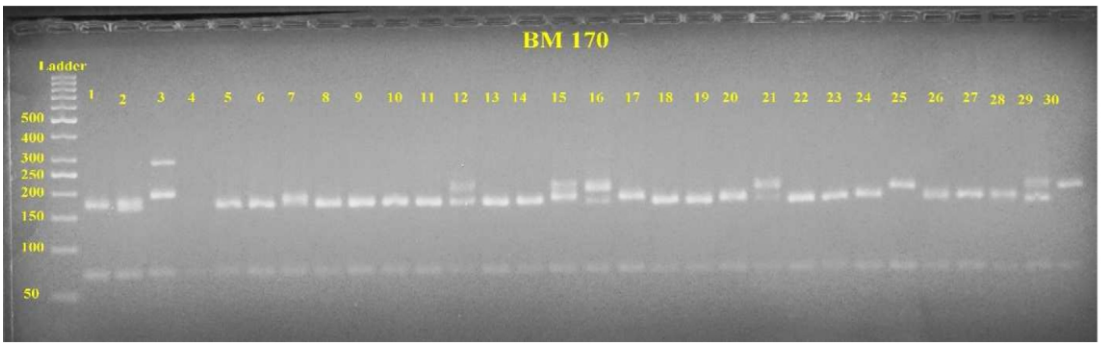
Various marker combinations were assessed to understand the DNA fingerprinting power of highly polymorphic markers. When a combination of three highly polymorphic markers *viz.*, CEDG 20, CEDG 44 and CEDG 243 were evaluated and it was observed that 21 genotypes produced unique profiles, whereas 23 genotypes produced unique profiles for a combination of

(A) CEDG 44





(B) BM 170



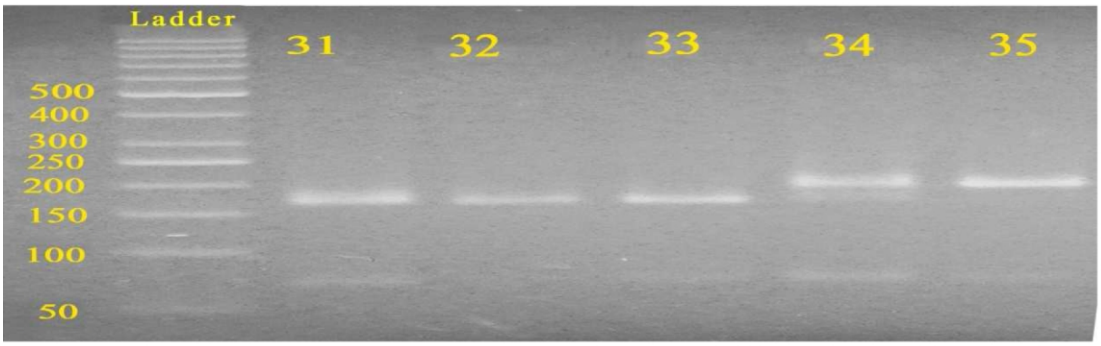


Plate.2. Representative gel profile of 35 blackgram genotypes using the primers CEDG 44 and BM 170

Table. 4.8. Number of alleles, allele frequency and PIC of 10 polymorphic SSR Markers

S.No	SSR Marker	Linkage Group	Number of alleles	Size of allele	Allele frequency	PIC
1	CEDG 006	2	3	113	0.89	0.21
2				136	0.06	
3				124	0.03	
4	CEDG 139	4	3	190	0.20	0.60
5				200	0.26	
6				216	0.54	
7	CEDG 20	5	6	207	0.03	0.79
8				242	0.17	
9				252	0.20	
10				270	0.34	
11				290	0.09	
12				300	0.11	
13	CEDG 173	9	2	125	0.49	0.56
14				141	0.46	
15	CEDG 180	10	3	143	0.14	0.61
16				150	0.49	
17				157	0.37	
18	CEDG 44	11	4	132	0.11	0.66
19				140	0.49	
20				148	0.26	
21				165	0.17	
22	BM 170	-	4	176	0.49	0.63
23				188	0.31	
24				200	0.09	
25				219	0.17	
26				290	0.03	
27	CEDG 198	-	4	203	0.60	0.56
28				219	0.14	
29				230	0.23	
30				250	0.06	
31	CEDG 225	-	2	125	0.60	0.48
32				135	0.40	
33	CEDG 243	-	3	205	0.43	0.65
34				215	0.34	
35				225	0.23	
	MINIMUM		**2**	**113**	**0.03**	**0.21**
	MAXIMUM		**6**	**290**	**0.89**	**0.79**
	AVERAGE		**3.4**	-	-	**0.57**

markers *viz.,* CEDG 20, CEDG 44 and BM 170. In contrast, the three marker combination involving CEDG 20, CEDG 44 and CEDG 198 produced unique profiles for 18 genotypes. Although, CEDG 243 had high PIC, BM 170 produced more unique combinations. This might be due to more number of loci (5) produced by BM 170 compared to CEDG 243 (3) which allowed differentiation of a greater number of genotypes or the less difference between the PIC of two markers. The marker CEDG 198 failed to differentiate more number of genotypes because of it's very low PIC (0.56) compared to CEDG 243 (0.647) and BM 170 (0.629). Hence, it can be concluded that high PIC is essential to differentiate more number of genotypes. However, if the difference between the PIC of markers is less it is essential to evaluate various combination of markers to identify the best combination that can differentiate more number of genotypes.

Likewise, the combination of four highly polymorphic markers *viz.*, CEDG 20, CEDG 44, CEDG 243 and BM 170 successfully differentiated 31 blackgram genotypes used in the present study, producing similar profile for the genotypes *viz.*, P-726 and VBG-11-6, RVSU-60 and LBG-645. The genotypes *viz.*, P-726 and VBG-11-6 were only differentiated by the markers CEDG 173 and CEDG 225. Among the two markers, CEDG 225 was able to differentiate both P-726 and VBG-11-6, RVSU-60 and LBG-645. Hence, the five marker combinations *viz.*, CEDG 20, CEDG 44, CEDG 243, BM 170 and CEDG 225 successfully differentiated all the 35 blackgram genotypes.

4.6.4 Molecular Profiling of 35 Blackgram Genotypes using 10 Polymorphic SSR Markers

Several factors can influence the ability to construct unique DNA fingerprint profiles, including the number of polymorphic markers and sample size used. Depending on the level of polymorphism of the markers used, larger the sample size, more the markers needed. In this study, all the genotypes were successfully fingerprinted with only five SSR loci, demonstrating the effectiveness of these SSR markers for fingerprinting of

blackgram genotypes. However, molecular profiles were generated using all the 10 polymorphic markers to improve the strength of the allele code (Table 4.9 a&b).

4.6.5 Development of QR Codes for 35 Blackgram Genotypes using Allele Code

A total of 10 polymorphic markers generated 35 polymorphic loci which produced unique codes for all the genotypes. Information regarding the molecular data could be made available in a single click using the Quick Response (QR) code. The breeder can have the information of type of allele produced by the particular marker in QR codes. Additionally, this QR barcode system has several advantages such as that it is a two-dimensional code that encodes text, numerical of 7089, 4296 alphanumeric characters and 2953 binary digits. Further, this system is fast and stores more information and can be read in 360° from any direction (Barghavi *et al.,* 2021). The codes can also be used as tags to the plants that can be scanned by mobile devices such as smart phones and tablets. Hence, this system can be used for varietal identification and benefit diverse researchers. Previously, Gao *et al.* (2012), Sohn *et al.* (2017) and Barghavi *et al.* (2021) have developed QR barcode system using the molecular markers for varietal identification in watermelon, soybean and rice respectively.

The QR codes were developed for all the 35 blackgram genotypes using online tool (www.barcode-generator.org) Fig. 4.8. To generate QR codes the allele codes of each blackgram genotype was pasted in the text box of the website, which directed to the option “Create QR Code”. By pressing on the option, a QR code image was generated which was saved in JPG format for further study.

Table . 4.9a. Molecular profiling of 35 blackgram genotypes using 10 polymorphic SSR markers

S.NO	ENTRIES	CEDG 006	CEDG 139	CEDG 20	CEDG 173	CEDG 180	CEDG 44	BM 170	CEDG 198	CEDG 225	CEDG 243	ALLELE CODE
1	**KDRS-136**	A	A	A	B	A	C	A	C	A	A	AAABACACAA
2	**SB-25-19**	A	C	B	B	C	C	A	C	A	A	ACBBCCACAA
3	**PU-205**	B	C	B	B	C	C	J	A	A	A	BCBBCCJAAA
4	**COBG-653**	N	B	B	A	C	B	N	C	A	C	NBBACBNCAC
5	**P-726**	A	C	D	B	B	C	A	A	A	A	ACDBBCAAAA
6	**VBG-11-6**	A	C	D	A	B	C	A	A	B	A	ACDABCAABA
7	**VBN-7**	A	C	D	A	A	B	B	C	A	B	ACDAABBCAB
8	**SB-40-5**	A	B	D	A	B	B	A	A	A	A	ABDABBAAAA
9	**TBG-104**	A	C	D	A	B	K	A	A	B	B	ACDABKAABB
10	**NDU-11-204**	A	C	D	A	B	C	B	A	A	B	ACDABCBAAB
11	**IPU-10-4**	A	B	B	N	B	C	A	B	B	B	ABBNBCABBB
12	**P-1032**	A	C	E	A	B	I	H	A	B	C	ACEABIHABC
13	**MBG-1058**	A	A	C	B	B	D	A	A	A	B	AACBBDAAAB
14	**PU-31**	A	C	E	A	A	C	A	A	B	B	ACEAACAABB
15	**VBG-10-010**	A	C	C	B	B	B	B	C	A	C	ACCBBBBCAC
16	**MBG-1061**	A	C	D	B	C	C	H	A	A	B	ACDBCCHAAB
17	**UG-708**	A	B	C	B	C	B	B	C	A	B	ABCBCBBCAB
18	**VBN-4**	A	C	F	B	B	B	A	A	B	B	ACFBBBAABB
19	**MBG-1051**	A	C	F	B	C	B	A	A	B	C	ACFBCBAABC
20	**TU-94-2**	B	A	D	B	B	A	B	B	B	B	BADBBABBBB
21	**P-728**	A	A	D	B	B	A	D	B	B	B	AADBBADBBB
22	**WBG-26**	A	C	B	B	B	A	B	B	B	C	ACBBBABBBC
23	**LBG-22**	A	B	E	B	C	A	B	B	A	C	ABEBCABBAC
24	**LBG-787**	A	B	B	B	B	D	B	D	A	B	ABBBBDBDAB
25	**LBG-752**	C	A	F	B	B	K	D	D	A	A	CAFBBKDDAA
26	**TU-67**	A	B	F	A	A	B	B	A	B	A	ABFAABBABA
27	**MBG-223**	A	B	C	A	A	B	B	A	A	A	ABCAABBAAA
28	**P-112**	A	B	D	A	C	I	B	C	A	C	ABDACBBCAC
29	**LBG-709**	A	C	D	A	N	B	H	A	A	A	ACDANBHAAA
30	**MBG-1050**	A	A	D	A	B	B	D	G	B	A	AADABBDGBA
31	**RVSU-60**	A	C	C	A	C	B	A	A	B	A	ACCACBAABA
32	**LBG-648**	A	C	N	N	C	B	A	A	A	A	ACNNCBAAAA
33	**LBG-645**	A	A	C	A	B	B	A	A	A	A	AACABBAAAA
34	**LBG-20**	A	C	N	A	C	D	C	A	B	C	ACNACDCABC
35	**VBG-11-31**	A	C	C	A	C	B	C	A	A	A	ACCACBCAAA

Table . 4.9b. Code key

CODE	CEDG 006	CEDG 139	CEDG 180	CEDG 198	CEDG 225	CEDG 44	BM 170	CEDG 173	CEDG 20	CEDG 243
A	113	190	143	203	125	132	176	125	207	205
B	136	200	150	219	135	140	188	141	242	215
C	124	216	157	230		148	200		252	225
D				250		165	219		270	
E							290		290	
F									300	
	G	**H**	**I**	**J**	**K**	**N**				
	AC	AD	BD	CE	CD	NILL				

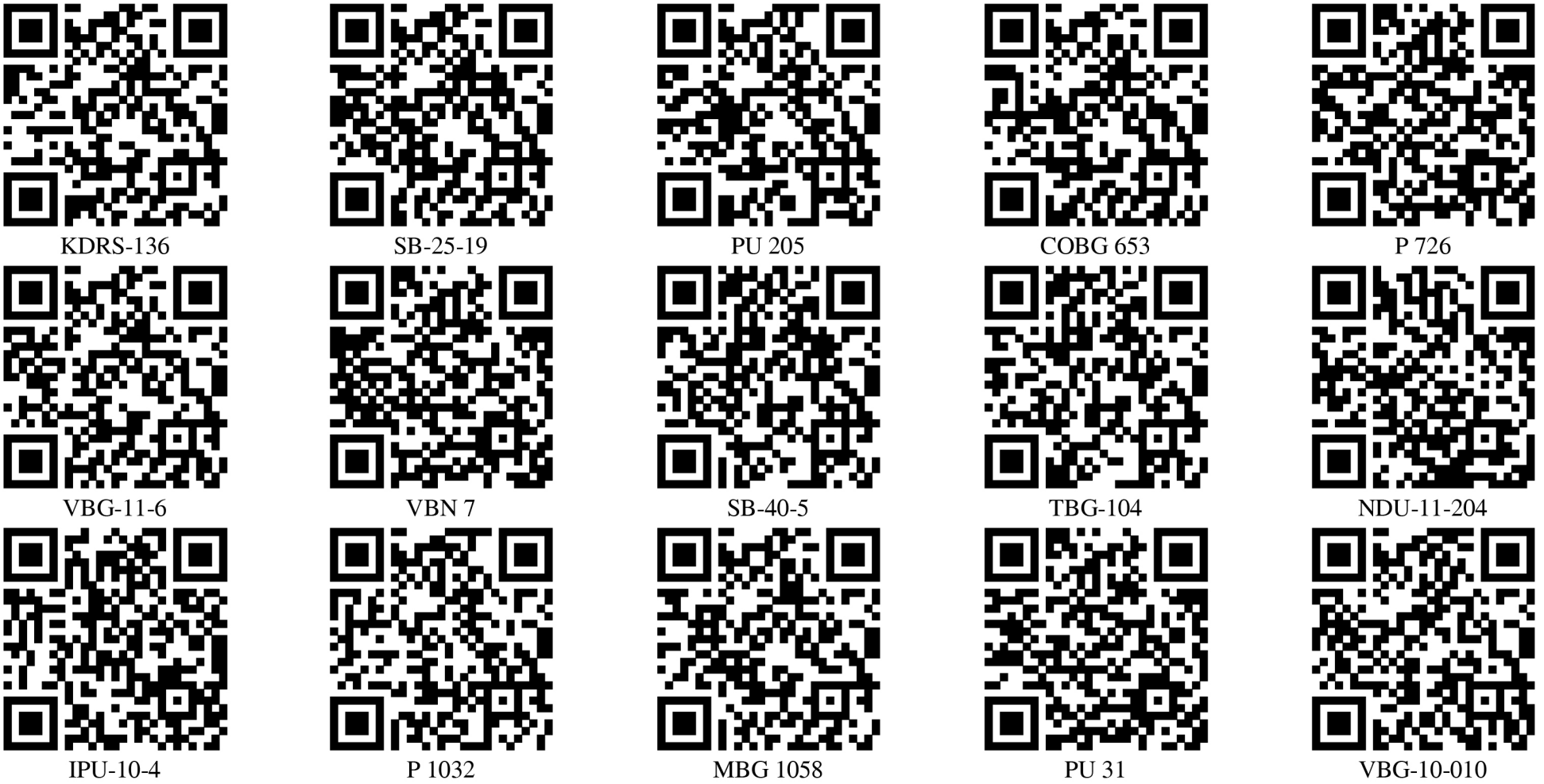


Fig. 4.8 QR codes generated for 35 blackgram genotypes


MBG 1061


UG 708


VBN 4


MBG 1051


TU-94-2

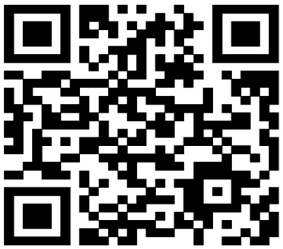

P 728


WBG 26

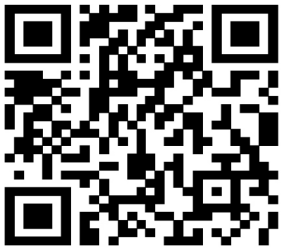

LBG 22


LBG 787


LBG 752

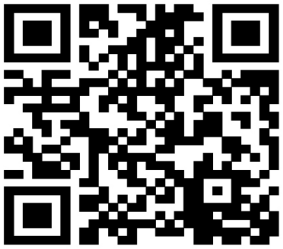

TU 67


MBG 223

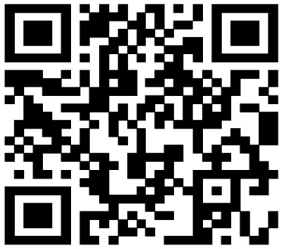

P 112


LBG 709


MBG 1050


RVSU 60


LBG 648


LBG 645


LBG 20


VBG-11-31

Chapter - V

Summary & Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

The present investigation entitled **"GT biplot analysis for yield traits and molecular profiling of selected lines in blackgram [*Vigna mungo* (L.) Hepper]"** was conducted to study the genetic parameters (variability, heritability and genetic advance), genetic diversity, genotype by trait biplot analysis for yield, yield contributing traits and to develop molecular profiles of 35 blackgram genotypes.

The field experiment was conducted at dryland farm of Sri Venkateswara Agricultural College, Tirupati, Acharya N.G. Ranga Agricultural University during *rabi* 2021-22 in RBD with three replications. The observations were recorded on randomly selected five plants in each genotype in each replication for plant height, number of primary branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length, hundred seed weight, seed yield per plant, and harvest index, while the data for days to 50% flowering and days to maturity were recorded on plot basis. The data thus generated were subjected to statistical analysis for the objectives under consideration.

Analysis of variance indicated significant differences among the genotypes for all the traits indicating the existence of sufficient variability in the material for all the traits under consideration. Mean performance of 35 blackgram genotypes revealed that the genotypes LBG 645, MBG 1058 and TU-94-2 were found to be the best genotypes they exhibited high mean values for most of the yield and yield traits, while the genotypes PU 31 and P 1032 had low *per se* for days to 50% flowering and days to maturity and high *per se* for few of the yield and yield attributing traits. Hence, these genotypes could be exploited in breeding programme to isolate suitable transgressive segregants for higher yield coupled with short duration.

High heritability coupled with high genetic advance as per cent of mean was recorded for number of primary branches per plant. Similarly, moderate heritability coupled with high genetic advance as per cent of mean was recorded for the trait number of clusters per plant. This specified the dominance of additive gene action in appearance of these traits. Therefore, simple selection might be fruitful for improvement of these traits in segregating generations.

Genetic divergence analysis using Mahalanobis D^2 statistics grouped 35 genotypes into 8 clusters. The distribution of genotypes into clusters was at random indicating that there was no association between geographical distribution and genetic diversity. Cluster I was the largest with nineteen genotypes followed by cluster III with nine genotypes and cluster V with two genotypes. While the clusters II, IV, VI, VII and VIII were solitary clusters consisting of one genotype each.

Inter cluster distances were greater than intra cluster distances indicating the existence of substantial diversity among the genotypes. The maximum inter cluster distance was observed between cluster IV and V followed by cluster V and VIII, cluster I and V, cluster V and VII and cluster VII and VIII indicating that the genotypes belonging to these clusters were genetically more divergent in that order. The genotypes in these clusters may serve as potential donors and crossing between these genotypes may result in novel recombinants. Among all the traits studied, days to 50% flowering followed by days to maturity, number of seeds per pod, number of primary branches per plant and pod length contributed relatively maximum towards the total divergence.

Intercrossing of genotypes from diverse clusters could be suggested to generate wide spectrum of variability and for ultimate selection in new breeding populations for improvement of yield attributes. By following this criteria, the crosses *viz.*, LBG 645 × P 112, (cluster V × cluster IV), LBG 645 × IP4-10-4 (cluster V × cluster VIII), LBG 645 × PU 31 (cluster V × cluster

I), LBG 645 × P 1032 (cluster V × cluster VII) and P 1032 × IPU-10-4 (cluster VII × cluster VIII) could be suggested for recombination breeding to get superior transgressive segregants with desirable yield contributing traits.

The genotype by trait (GT) biplot analysis was used to identify the best traits that are important to identify superior genotypes and to know the relationships between traits and genotypes based on overall pattern of data. Among the 35 tested genotypes, seed yield was positively correlated with number of pods per plant, number of clusters per plant, number of primary branches per plant, number of seeds per pod, plant height, days to maturity, pod length, number of pods per cluster and harvest index. However, seed yield was negatively correlated with days to 50 % flowering and 100 seed weight.

Based on their discriminating ability and representativeness, the trait number of primary branches per plant was considered as best followed by number of clusters per plant, plant height and number of seeds per pod. Hence, these traits could be used to identify the superior genotypes. The genotype LBG 645 was identified as the ideal cultivar followed by KDRS 136, MBG 1058, LBG 648, P 1032, LBG 20 and NDU-11-204 as they registered high mean performance. Therefore, these genotypes could be utilized as potential donors in breeding programs aimed at improving yield and yield traits.

Molecular profiling of 35 blackgram genotypes revealed that, out of 16 SSR markers, ten were polymorphic and generated unique DNA profiles for 35 blackgram genotypes studied. A total of 35 alleles were generated and frequency of these alleles ranged from 0.03 to 0.89. Three unique alleles were found with markers CEDG 006, CEDG 20 and BM 170 for the genotypes LBG-752 (124bp), KDRS-136 (207bp) and PU-205 (290) respectively.

PIC values in polymorphic markers ranged from 0.21 (CEDG 006) to 0.79 (CEDG 20) with 0.57 as average. Based on the level of polymorphism detected by individual marker, four SSR markers (CEDG 20, CEDG 44,

CEDG 243 and BM 170) were most informative, which displayed very high PIC values. This set of markers had the enormous potential to identify most of the blackgram genotypes. Further, based on observation of marker combinations, the combination of five markers including four most informative SSR markers (CEDG 20, CEDG 44, CEDG 243, BM 170) along with the marker CEDG 225 successfully produced unique profile for all the 35 blackgram genotypes. However, molecular profiles were generated using all the 10 polymorphic markers to improve the strength of the allele code.

FUTURE LINE OF WORK

- Based on *per se* performance the genotypes LBG 645, MBG 1058 and TU-94-2 were adjudged as best genotypes for yield and yield attributes, while the genotypes PU 31 and P 1032 were best for early maturity traits. Hence, these genotypes could be utilized in future breeding programme for development of short duration varieties coupled with high seed yield in blackgram.
- The genotypes from diverse clusters with high *per se* and complementarity for traits of interest could be used as parents for future hybridization programme to create a broad spectrum of variability in segregating generations.
- The SSR markers *viz.*, CEDG 20, CEDG 44, CEDG 243 and BM 170 were found to have high discriminative power. Hence, this set of markers can be used in future programmes to identify most of the blackgram genotypes.
- The present study on molecular profiling was done with limited number of SSR markers. However, comprehensive studies that include exhaustive sampling of all registered blackgram genotypes for a country or a region and more markers are necessary for evaluation of the feasibility of using DNA fingerprinting in the management of registered blackgram genotypes.

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Appendices

APPENDIX A

EQUIPMENTS USED

1. Agarose gel electrophoresis system (Biorad)
2. Autoclave
3. DNA thermal cycler (Eppendorf and Bioradmaster cycler gradient)
4. Freezer -20 °C (Vestfrost)
5. Gel documentation system (Bio-rad)
6. Mini cooler of -20 °C (Tarsons)
7. Magnetic stirrer (Wisd)
8. Microwave oven (Samsung)
9. Centrifuge (Eppendorf 5804R)
10. Mini centrifuge (Tarsons)
11. Nanodrop (ND 100)
12. pH meter (Eutech instruments)
13. Water bath (Wisd and Polyscience)
14. Weighing balance (Unibloc)
15. Thermo mixer (Eppendorf)

APPENDIX B

LIST OF CHEMICALS

1. Agarose (Genei)
2. 6X loading dye (Thermo Scientific)
3. Boric Acid (Avra)
4. Chloroform (Thermo Scientific)
5. CTAB (Himedia)
6. dNTPs (Deoxy nucleotide triphosphates) (Genei)
7. EDTA (Ethylene Diamino Tetra Acetic acid) (Thermo Scientific)
8. Ethidium bromide (Sigma)
9. Ethylalcohol (Changshu Hongsheng Fine Chemicals)
10. Isoamyl alcohol (Qualigens)
11. Isopropanol (Emplura)
12. β −Mercaptoethanol (Thermo Scientific)
13. NaCl (Sodium chloride) (Thermo Scientific)
14. NaOH (Sodiun hydroxide) (Qualigens)
15. Phenol (Thermo Scientific)
16. Poly vinyl pyrrolidone (Thermo Scientific)
17. *Taq* polymerase (Genei)
18. Tris base (Himedia)
19. 100bp ladder (Thermo Scientific)
20. *Taq* buffer with$MgCl_2$ (Genei)
21. Primers (Sigma)

APPENDIX C

PREPARATION OF BUFFERS AND STOCK SOLUTIONS

DNA Extraction Buffer

Component	Quantity
2 % (w/v) CTAB (Nalgene)	10g
100 Mm Tris HCl, pH 8.0	100 ml of 0.5 M Tris HCl
20 mM EDTA, pH 8.0	20 ml of 0.5 M EDTA
1.4 M NaCl	140 ml of 5 M NaCl
1% PVP	5 g
β −Mercaptoethanol	290 µl

All the above ingredients except CTAB were added in respective quantities and final volume was made upto 500 ml with double distilled water, the solution was autoclaved. Then it was allowed to attain room temperature and 10 g of CTAB was dissolved by intense stirring, stored at room temperature.

Tris (1M) 100 ml

To prepare 1M Tris, 12.114 g of Tris was dissolved in 100 ml of distilled water

Nacl (5M) 100 ml

NaCl (29.22 g) was dissolved in 100 ml of distilled water.

EDTA (0.5M) 100 ml

EDTA (18.612 g) was dissolved in 50 ml of distilled water by adding 4 g of NaOH pellets. Solution was stirred by adding another 20 ml of water and EDTA was allowed to dissolve completely. Later pH was adjusted to 8 by adding 2N NaOH drop by drop and made the volume to 100 ml by adding distilled water.

1% PVP:

One gram of PVP was dissolved in minimal quantity of water and made upto 100 ml with millipore water.

Phenol: Chloroform: Isoamyl alcohol (25:24:1)

Equal parts of equilibrated phenol and Chloroform: Isoamyl alcohol (24:1) were mixed and stored at 4 ˚C.

Absolute ethanol - Stored at 4 ˚C

70% Ethanol

Absolute ethanol - 70 ml

Distilled water - 30 ml

90 % ethanol

Absolute ethanol - 90 ml

Distilled water - 10 ml

AGAROSE GEL ELECTROPHORESIS:

10X TBE Buffer, pH 8.3

Tris base-108 g

Boric acid-55 g

EDTA-9.3 g

Dissolved in 1000 ml amount of distilled water.

Composition of loading dye:

Glycerol-2 ml

10xTBE-600 µl

1% BPB (Saturated)-2 ml (0.1 g in 10 ml of distilled water)

10% SDS-600 µl

0.5M EDTA-500 µl

Double distilled water-300 µl

Ethidium bromide preparation:

Ethidium bromide-10 mg

Sterile distilled water-1.0 ml

3M Sodium acetate (pH-5.2)

sodium acetate (14.6 g) was dissolved in 70 ml of distilled water and pH was adjusted to 5.2 by using glacial acetic acid. Then, the volume was made to 100 ml by adding distilled water.