Assessment of Genetic Variability and Trait Associations in Chickpea (*Cicerarietinum* L.) Germplasms for Targeted Breeding Strategies

ABSTRACT

Chickpea (*Cicer arietinum* L.) is a pivotal legume crop with substantial contributions to global food security and agricultural sustainability due to its high protein content, adaptability and nitrogen-fixing ability. The efficiency of breeding programs in enhancing chickpea productivity and resilience relies heavily on understanding and utilizing genetic variability within the germplasm. This study investigates the extent of genetic variability among forty-three chickpea genotypes, focusing on key traits such as number of pods (NPP), biological yield (BY), harvest index (HI), days to flowering (DTF), days to maturity (DTM), that influence yield and other agronomic characteristics. Analysis of variance showed significant variability among genotypes for various traits. Higher phenotypic coefficients of variation (PCV) compared to genotypic coefficients (GCV) indicated the presence of environmental influence, particularly for traits like number of pods per plant (NPP) and biological yield (BY). High heritability was observed for days to flowering (DTF) and maturity (DTM), while genetic advance as percent of mean (GAM) varied, indicating additive gene action for some traits. Correlation and path analysis revealed positive relationships between plot yield and traits such as Days to Flowering (DTF), plant height (PH), and harvest index (HI). Principal component analysis (PCA) and cluster analysis indicated significant genetic diversity, grouping genotypes into clusters and identifying top performers like GJG1801 and NBEG 924. This study emphasizes the importance of genetic variability in breeding programs, aiding in the selection of elite genotypes for targeted traits improvement.

INTRODUCTION

Chickpea (*Cicer arietinum* L.), one of the most vital legume crops globally, plays a significant role in ensuring food security and agricultural sustainability. With its high protein content, adaptability to various climatic conditions, and ability to fix atmospheric nitrogen, chickpea stands out as a crucial crop for both human nutrition and soil health (Maesen, 1972). Despite its importance, chickpea production faces numerous challenges, including biotic and abiotic stresses that significantly limit the yield potentiality (Kushwah *et al.,* 2020). Therefore, enhancing chickpea productivity and resilience through genetic improvement is a primary objective of contemporary agricultural research. Genetic variability, the cornerstone of plant breeding, refers to the diversity in gene frequencies within a species. Morphological and biometrical characterisation of the germplasm as a part of pre breeding program can boost the success of developing potential varieties for widespread cultivation (Nandedkar *et al.,* 2021)

Understanding the extent and nature of genetic variability within chickpea germplasm is fundamental for effective breeding programs (Singh *et al*., 2020, Philanim *et al., 2024*). Key metrics used to assess genetic variability include phenotypic coefficient of variation

(PCV) and genotypic coefficient of variation (GCV). PCV and GCV provide insights into the extent of phenotypic and genotypic variability, which are crucial for determining the potential tools for selection in breeding programs (Sharma *et al*., 2019). Heritability is another critical parameter, measures the proportion of observed variation that can be attributed to genetic factors. High heritability estimates indicate that traits are likely to respond well to selection, thereby facilitating genetic improvement (Kumar *et al*., 2018). Correlation and path analysis are employed to understand the relationships between different traits, which helps in identifying key traits that directly or indirectly affect yield. Correlation analysis provides information on the strength and direction of associations between traits, while path analysis partitions these correlations into direct and indirect effects, offering a more nuanced understanding of trait interactions (Reddy *et al*., 2017). These analyses are instrumental in selecting traits that can be targeted for simultaneous improvement through hybridization. Cluster analysis, a multivariate technique, groups genotypes based on their similarity across multiple traits, thereby identifying distinct genetic clusters within the germplasm. This method helps in identifying genetically diverse parents that can be used in hybridization programs to create high-yielding and resilient varieties (Patil *et al*., 2016).

By exploring the genetic diversity through cluster analysis, breeders can efficiently utilize the available genetic resources. This research aims to provide a comprehensive analysis of genetic variability in chickpea, focusing on both phenotypic and genotypic diversity. By employing a combination of field trials, molecular marker analysis and advanced statistical methods, we seek to elucidate the patterns of genetic variation within a diverse set of chickpea accessions. The findings from this study are expected to contribute to the development of superior chickpea cultivars for commercial cultivation, thereby enhancing crop productivity and sustainability.

MATERIALS AND METHODS

Experimental materials:

The current experiment comprising of 43 chickpea genotypes and it was conducted at Research cum Instructional farm, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, during *Rabi* 2020-21.The details of the genotypes under study are elucidated in table 1.

Experimental design:

The genotypes were planted in a randomised block design (RCBD) with three replications with a spacing of 22.5 cm x 10 cm. Each plot comprised of 4 rows of 4m length per genotype per replication. The row x row and plant x plant distance of 22.5 cm and10 cm. Recommended package of practices were followed to raise the crop . Eleven biometrical traits viz. days to 50% flowering (DTF), Days to maturity (DTM), Plant height (cm) (PH), Number of primary branches (NPB), Number of secondary branches (NSB), Number of pods per plant (NPP), Number of seeds per pod (NSP), Biological yield (g) (BY), Harvest index (%) (HI), Hundred seed weight (g) (HSW) and plot yield (g) (PLYG). All observations were recorded on five randomly selected plants per genotype.

Statistical analysis:

Analysis of Variance (ANOVA) was employed to estimate genetic variability, and determine the significance of differences among group means for the traits studied, following the methodology outlined by Fisher (1952). The phenotypic (PCV) and genotypic coefficients

of variation (GCV) for the traits was calculated using the formula provided by Burton and De Vane (1953). The traits were categorized based on CV values into high (>20%), moderate (10%-20%), and low (<10%) variation. Broad-sense heritability was assessed following the formula of Hanson *et al.* (1956) and categorized as low, moderate, or high based on the criteria established by Johnson *et al.* (1955). The expected genetic advance (GA) was determined using the approach outlined by Johnson *et al.* (1955). Correlation analysis was performed according to the methodology described by Miller *et al.* (1958). Path analysis (Wright, 1921; Dewey and Lu, 1959), was used to partition the correlation coefficients into direct and indirect effects of independent variables on the dependent variable to study the effects of different traits on yield. Principal Component Analysis (PCA), a dimension reduction technique, was conducted following the methods described by Massey (1965) and Jolliffe (1986). Cluster analysis was done based on ward's D^2 method using Euclidean distances (Franco *et al.*, 1997).

Software:

All the analysis was carried out using Rstudio version 402 (Posit team, 2024) and metan package (Olivoto and Lúcio, 2020). The plots were generated using Origin software (OriginLab Corporation, 2024).

RESULTS AND DISCUSSION

Analysis of variance revealed that all the traits studied showed significant variances at genotypic level, indicating the presence of ample amount of variability amongst the genotypes. Similar results were reported by Gediya *et al.*, (2019), Aslam *et al.*, (2020) and Saxena *et al.*, (2021). The mean values of the genotypes for all the traits are elucidated in table 2.

Performance of germplasms to different traits

The flowering periods ranged between 48 days to 68 days with an average flowering time of 60 days, while days to maturity ranged between 90 days to 110 days with an average of 100 days for maturity (table 3). Plant height ranged between 34.13 cm to 63.93 cm with an average height of 52.2 cm, reflecting differences in growth potential among the genotypes. The number of primary branches ranged between single branch to three branches with an average of two primary branches, while the secondary branch number ranged between 4 to 17 branches with an average of ten branches. Number of pods per plant ranged between 20 to 90 pods with a mean pod number of 43 pods, while number of seeds per pod ranged between a single seed to two seeds per pod with an average of one seed per pod. The biological yield ranged from 6.46 to 37.93 g per plant, with an average of 16.60 g, while harvest index (HI) ranged from 32.78% to 80.30%, with a mean of 56.46%. The hundred-seed weight (HSW) varied between 14.00 and 36.33 g, with a mean of 21.92 g, showing variability in seed size. Finally, the plant yield (PLYG) ranged widely from 61.00 to 1045.00 g, with an average of 676.75 g, reflecting substantial differences in overall productivity among the genotypes. These results highlight the potential for selecting genotypes with desirable traits for breeding programs aimed at improving chickpea productivity.

Genetic variability

The Phenotypic coefficient of variation (PCV) and Genotypic coefficient of variation for different traits are illustrated in Fig 1 and presented in table 3. The PCV values were higher than the GCV indicating the presence of environmental influence in expression of

traits. High PCV and GCV values were exhibited by NPP, BY, HSW and PLYG. While high PCV and moderate GCV were observed for NPB, NSP, and HI. DTF and DTM exhibited low values for PCV and GCV. Similar results were reported by Dar *et al.,* (2020), Rathod *et al.,* (2020) and Datta *et al.,* (2023).

Heritability (h^2_{bs}) and Genetic advance as percent of mean (GAM):

Heritability is defined as the ratio of genotypic variance to phenotypic variance or total variance and it is usually presented in percentages. The estimates of heritability help the plant breeder in selection of elite genotypes from the wide range of diverse genetic populations. On the other hand, GAM gives an idea about genetic gain after selection on certain trait. Heritability together with the GAM provides information to forecast the genetic gain under selection as compared to only heritability estimates. High heritability values were expressed by DTF and DTM. Wheras, PLYG and HSW exhibited moderate heritability and rest of the other traits exhibited low heritability values (fig 2 and table 3). High GAM values were observed for PLYG, NPP, HSW, BY and NSB while rest of the traits exhibited either medium or low levels of GAM. High values of heritability and GAM indicates that the expression of the trait is governed by additive gene action and the selection of such traits is more efficient. Heritability (broad sense) with moderate or high value along with low estimate of GAM or vice versa hinted the involvement of non-additive type of gene action. The researchers Gizachew *et al.* (2020), Kumar *et al.* (2020), Rathod *et al.* (2020) and Jha *et al.,* (2023) showed that moderate to high values of heritability with greater magnitude of genetic advance as mean percentage suggesting the predominance of role of additive genes and selection pressure may be implemented efficiently.

Correlation and Path analysis:

Correlation studies are essential in plant breeding as they help identify relationships between traits, enabling breeders to select and enhance desirable traits effectively (Robinson *et al.,* 1951 and Johnson *et al.,* 1955). Plot yield exhibited positive correlation with DTF, PH, NSP and HI (Fig 3). Negative correlations with plot yield were observed for the traits DTM, NSB, BY, and NPP. Even though number of pods per plant is a yield attributing trait, the negative correlation with plot yield can be attributed to ill filled pods or empty pods. Biological yield exhibited positive correlation with DTF, DTM, NSB, NPP, NPB, PH, NSP and HSW. Dar et al. (2020), Kumar et al. (2020), Mengitsu *et al.* (2020), Rathod *et al.* (2020) and Saxena *et al.,* (2023) have reported comparable associations amongst these traits with plot yield.

Path analysis provides elucidates the underlying complex interactions amongst the independent traits which influence their effect on the dependant trait. In this scenario, plot yield (g) was chosen as the dependant trait and the interactions are visualised (Fig 4). NPP, NSP, PH HI, NPB, DTF showed positive direct effects, while BY, DTM, HSW and NSB showed negative direct effects. Similar results were reported by Mihoariya *et al.,* (2023). BY exhibited positive indirect effects through NPP which also bolsters the reason for negative correlation of NPP with plot yield. Majority of the traits exhibited negative indirect effects through BY except HI.

Principal Component Analysis and Cluster analysis:

Principal component analysis is a dimension reduction technique which can be used to quantify the amount of genetic diversity present in the available set of genotypes (Bhusal, 2016). The PCA resulted in formation of twelve PCs, out of which four PCs showed an Eigen value of >1. The cumulative proportion of variance explained by these four PCs is 77.34% with PC_1, PC_2, PC_3, PC_4 explaining 33.84%, 18.42%, 13.12% and 11.96% respectively (Fig 5).Correlation between the variables and PC_s showed that plot yield (g) is positively correlated with PC_4, while HSW and PH with PC_3, while DTF, DTM, NSB, BY were positively correlated with PC_1 (Fig 6). The PCA biplot plotted the genotypes with the traits as vectors and the scattering of genotypes across the biplot represents the amount of diversity present between the genotypes (Fig 7). Cluster analysis based on Euclidean distances and ward D^2 method has resulted in grouping of the genotypes into six clusters (Fig 8). The genotype RVSSG-92 alone was placed into one cluster while the largest cluster consisted of fourteen genotypes.

Conclusion:

The success of any breeding program heavily relies on the presence of variability in the germplasm used. The study of different variability parameters can help us in elucidating the extent of diversity present in the germplasm. The association analysis and path analysis aids us in in understanding the trait relationships for targeted trait improvement programs. PCA and Clustering groups the genotypes into different clusters based on their diversity, which helps us in selection of diverse parents for hybridization programs. Based on the plot yield (g), genotypes GJG1801, NBEG 924, GL15026, BDNG 2018-8, PHULEG1210-1, RSGD-1116, NBEG 934, IG 2020-55, RKG 13-21, GJG 1803 were found to be the top ten best performers. These genotypes can be further used in hybridization programs to develop new breeding material asual as high yielding varieties for commercial cultivation.

Table 1: List of forty-three genotypes used in the study for assessing genetic diversity

S. No.	Entry name	S.No.	Entry name
1	JG 16	23	GL 16056
2	RG 2016-50	24	RVSSG-91
3	GL15026	25	PHULEG1210-1
4	BDNG 2017-1	26	JG 2020-03
5	GJG 1803	27	RVSSG-92
6	H 16-22	28	BG 4020
7	H 05-24	29	NDG 19-1
8	RSGD-1116	30	KCD 2019-5
9	GNG2513	31	PG 266
10	PHULE G191111	32	IPCB 2016-25
11	BAUG 109	33	GNG2517
12	DBGC-3	34	RKG 13-21
13	JG 2020-56	35	GJG1801
14	KCD 2019-7	36	AKG-1702
15	PBC 584	37	IPC 2015-48
16	BG 4021	38	NBEG 934
17	BDNG 2018-8	39	NBEG 924
18	PG 265	40	RSGD-1117
19	INDIRA CHANA 1	41	JG 315
20	JG 2020-55	42	IPC 2014-133
21	JAKI 9218	43	RKG 13-62
22	IG 2020-04		

Table 2: Analysis of variance of forty-three genotypes for eleven traits

SV	DF	Mean Sum of Squares										
		DTF	DM	PH	NPB	NSB	NPP	NSP	BY	HI	HSW	PLYG
Treatment	42	74.60**	66.65**	111.50**	0.54**	26.83**	675.20**	0.20**	109.93**	317.34**	69.97**	114220.19**
Replication	2	0.84	3.93	7.56	0.08	7.80	55.24	0.03	15.57	0.083	21.87	16071.77
Error	84	1.08	3.15	31.45	0.02	10.64	228.57	0.15	44.97	1.148	11.22	14046.27

*and ** Significant at 5% and 1% probability level, DTF = Days to 50% flowering; DM = Days to maturity; PH = Plant height (cm); NPB = No. of primary branches; NSB = No. of secondary branches; NPP = No. of pods/plant; NSP = No. of seeds/pod; BY = Biological Yield; HI = Harvest Index (%); HSW = Hundred seed weight (g); PLYG = Plot yield (g)

Table 3: Variability parameters of eleven studied traits

	DTF	DTM	PH	NPB	NSB	NPP	NSP	BY	HI	HSW	PLYG
Min	48.00	90.00	34.13	1.00	4.00	20.00	1.00	6.46	32.78	14.00	61.00
Max	68.00	110.00	63.93	3.00	17.00	90.33	2.13	37.93	80.30	36.33	1045.00
Mean	59.84	100.50	52.20	2.00	10.00	42.95	1.28	16.60	56.46	21.92	676.75
PCV	8.65	5.16	14.44	23.37	29.63	36.51	24.90	37.61	20.89	24.39	31.51
GCV	8.24	4.39	9.78	19.59	28.74	33.51	19.77	35.32	18.33	20.54	26.86
H^2_{bs}	90.79	72.33	45.93	70.25	94.12	84.20	63.05	88.23	76.97	70.87	72.65
GAM	16.18	7.69	13.66	33.83	57.44	63.34	32.34	68.36	33.13	35.62	47.16

Min = Minimum; Max: Maximum; PCV: Phenotypic coefficient of variance; GCV = Genotypic coefficient of variance; H^2_{bs}; GAM = Genetic advance as percent of mean (%); DTF = Days to 50% flowering; DM = Days to maturity; PH = Plant height (cm); NPB = No. of primary branches; NSB = No. of secondary branches; NPP = No. of pods/plant; NSP = No. of seeds/pod; BY = Biological Yield; HI = Harvest Index (%); HSW = Hundred seed weight (g); PLYG = Plot yield (g)

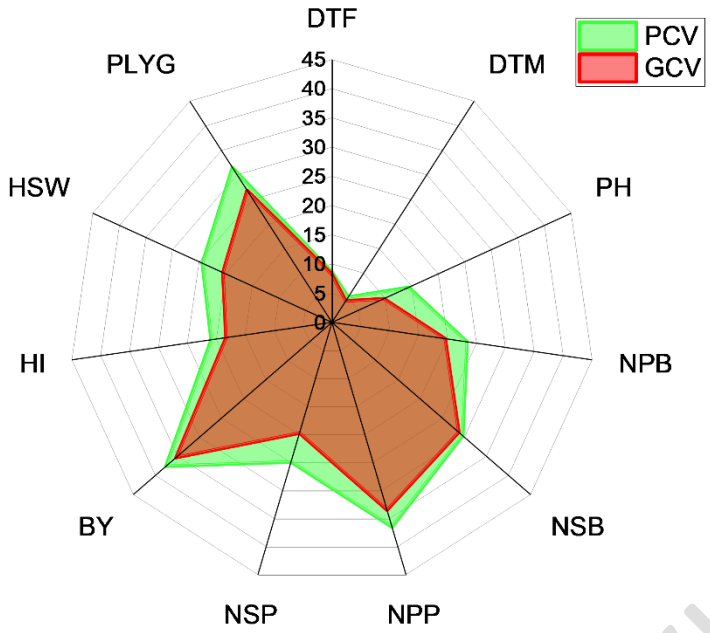

Fig 1: Phenotypic and genotypic coefficients of variation for eleven traits studied. DTF = Days to 50% flowering; DM = Days to maturity; PH = Plant height (cm); NPB = No. of primary branches; NSB = No. of secondary branches; NPP = No. of pods/plant; NSP = No. of seeds/pod; BY = Biological Yield; HI = Harvest Index (%); HSW = Hundred seed weight (g); PLYG = Plot yield (g)

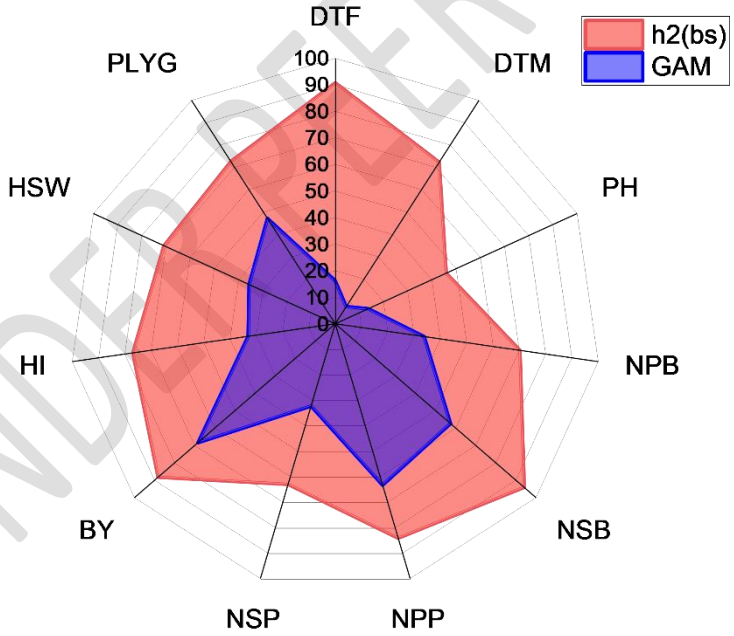

Fig 2: Heritability (h^2_{bs}) and genetic advance as mean (GAM) for eleven traits studied. DTF = Days to 50% flowering; DM = Days to maturity; PH = Plant height (cm); NPB = No. of primary branches; NSB = No. of secondary branches; NPP = No. of pods/plant; NSP = No. of seeds/pod; BY = Biological Yield; HI = Harvest Index (%); HSW = Hundred seed weight (g); PLYG = Plot yield (g)

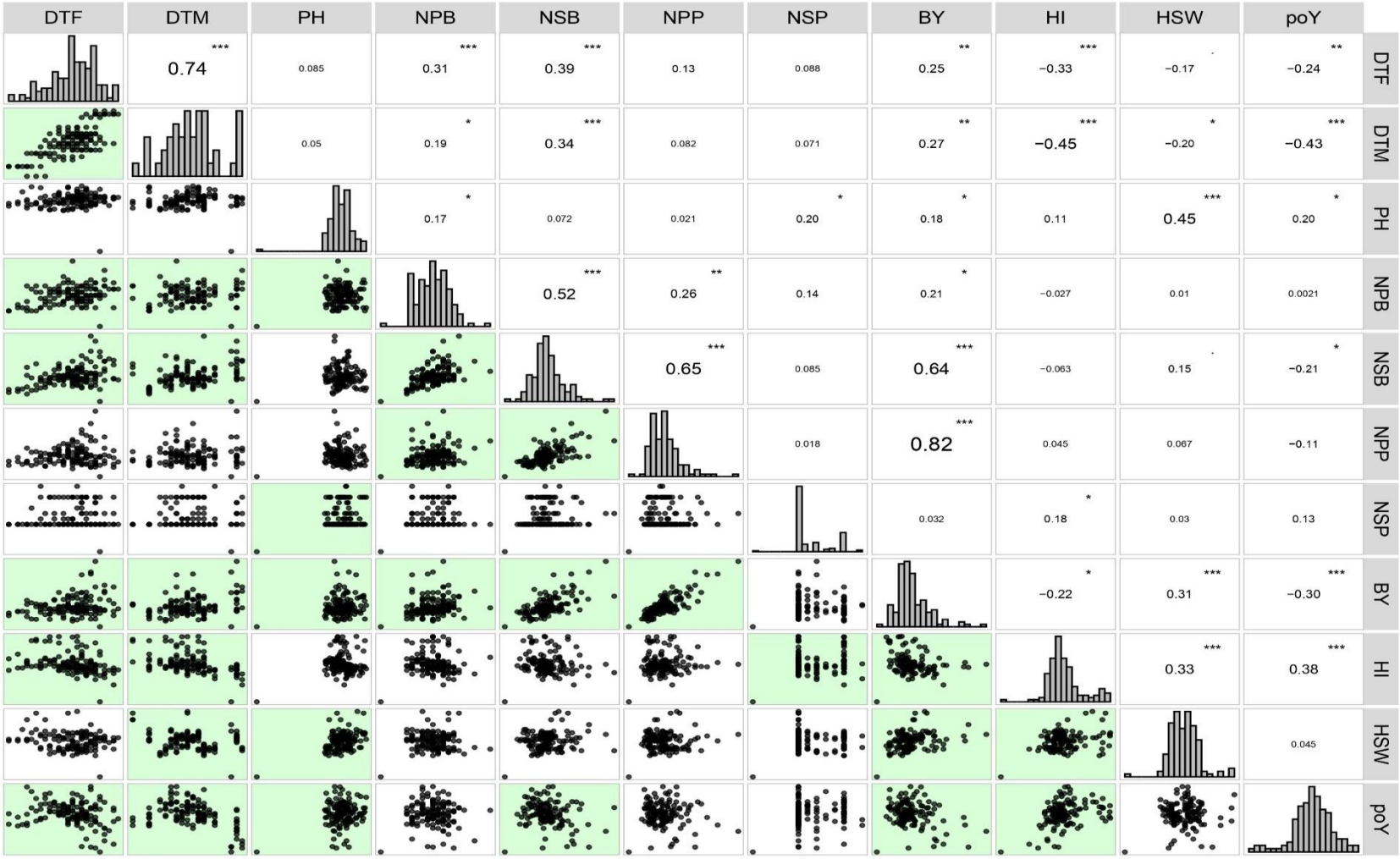

Fig 3: Correlation plot between the studied traits. The lower diagonal shows the scatter plot of the genotypes and the diagnol shows the population distribution as histograms

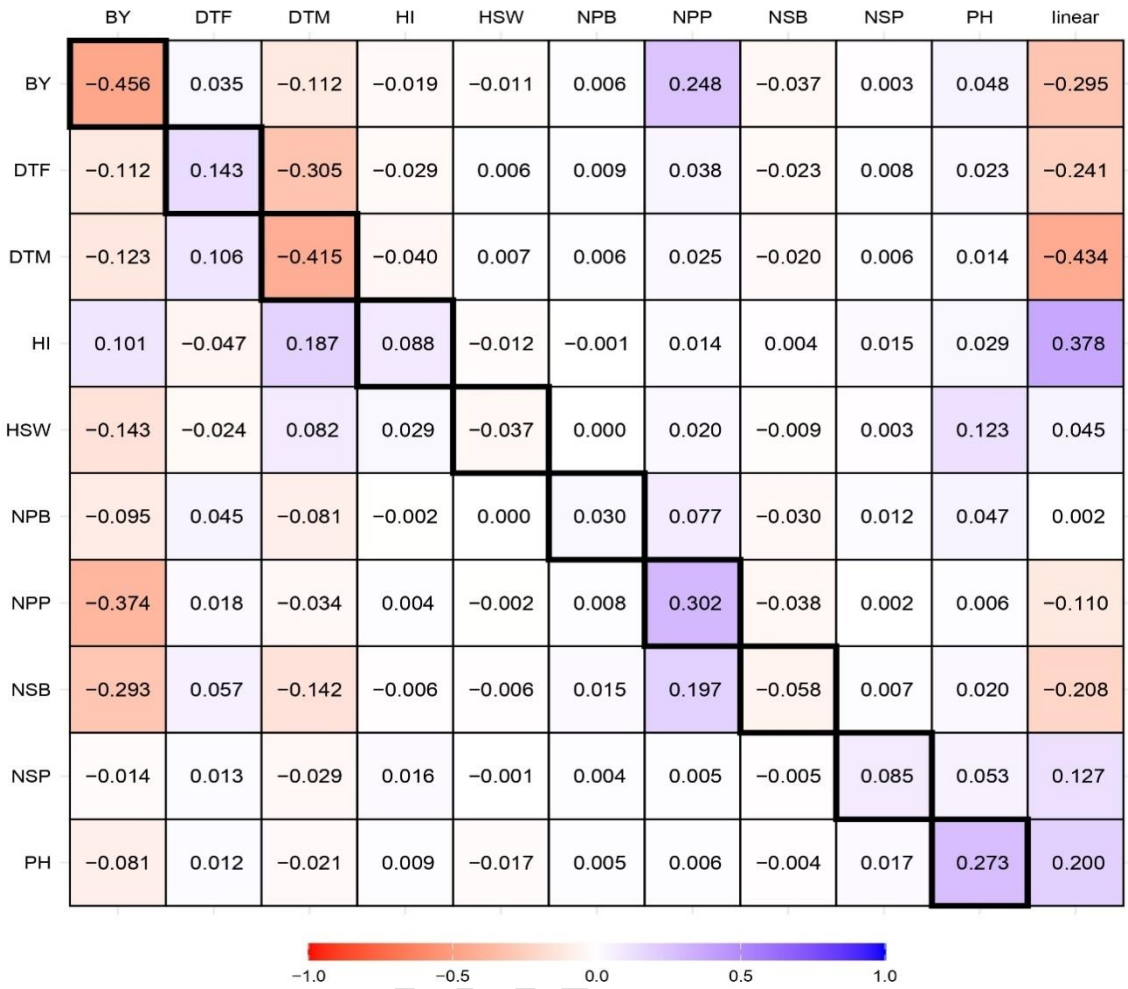

Fig 4: Path coefficient analysis with plot yield (PLYG) as the dependant variable. The diagonals represent the direct effects while the top and bottom cells indicate the indirect effects.

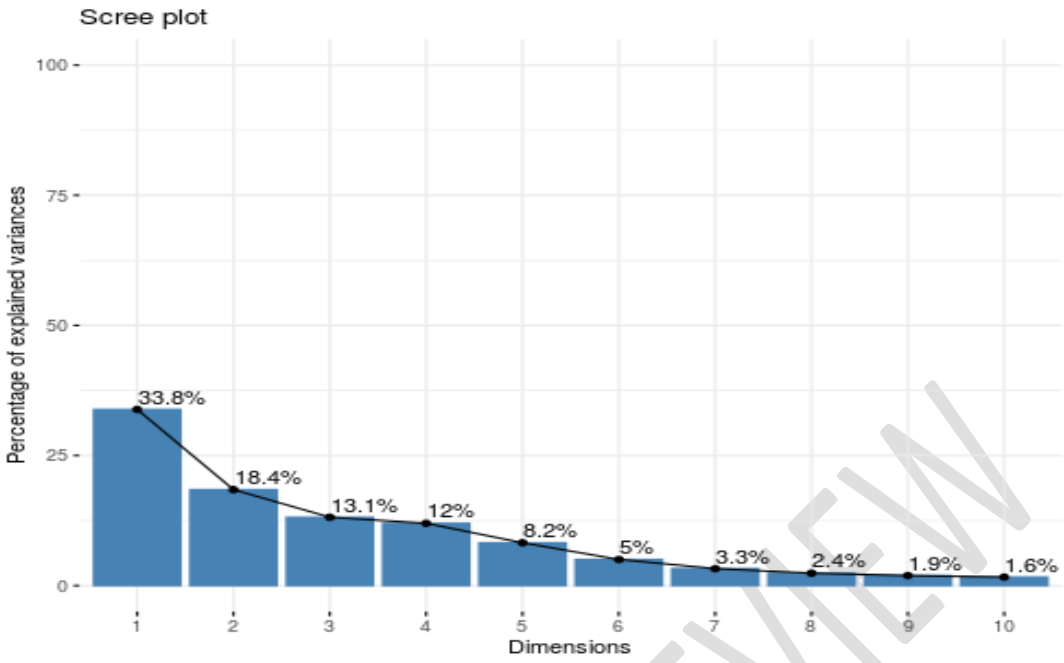

Fig 5: Scree plot showing the percent of variation explained by different principal components

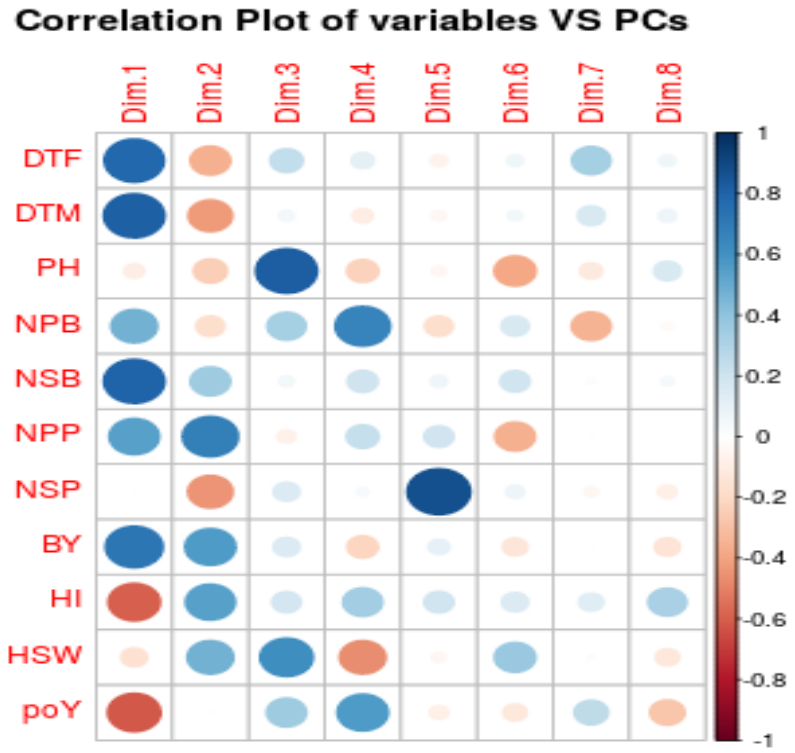

Fig 6: Correlation between different variables and PCs

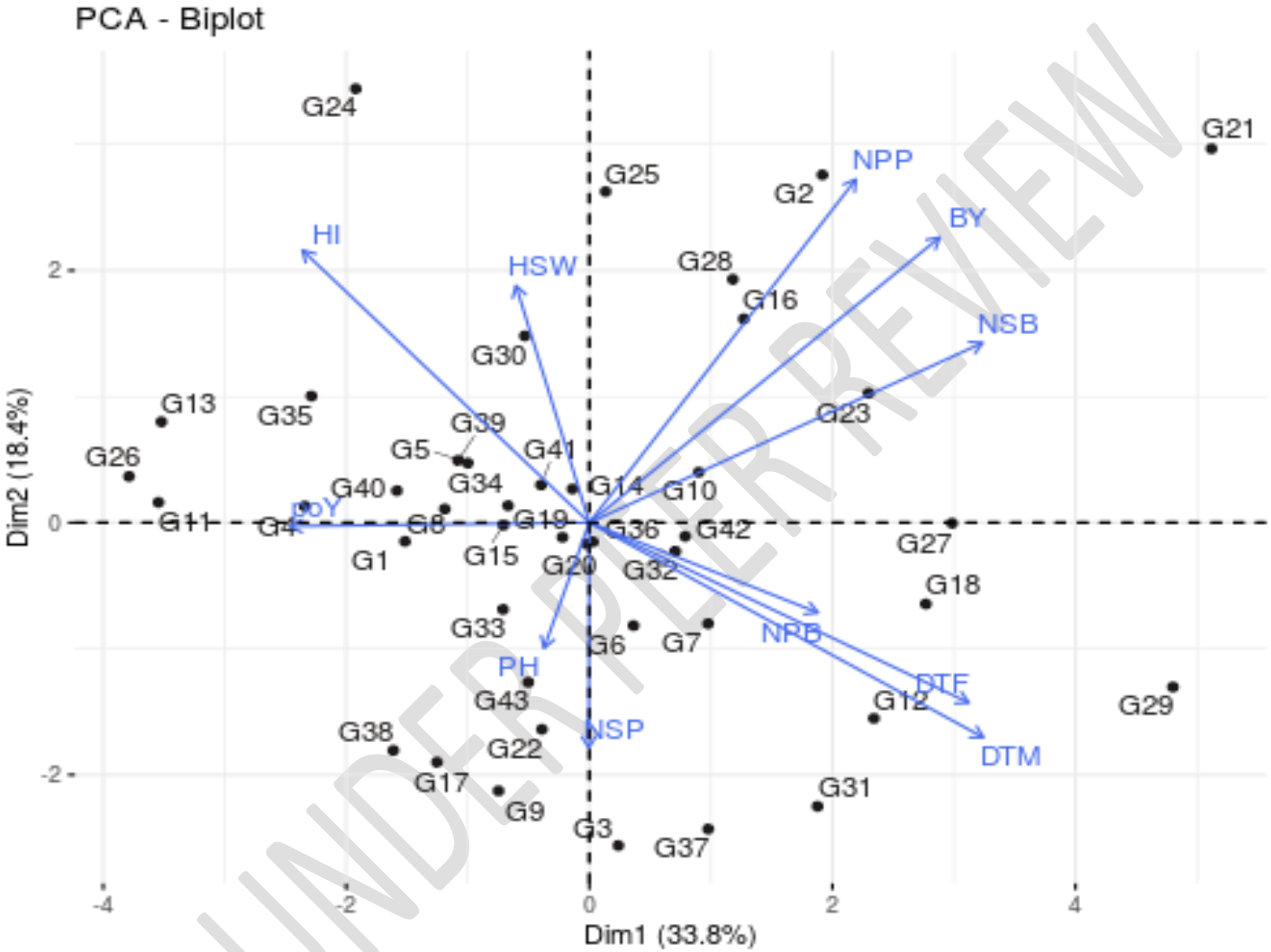

Fig 7: PC_1 vs PC_2 biplot with genotypes plotted across the biplot and traits as the vectors

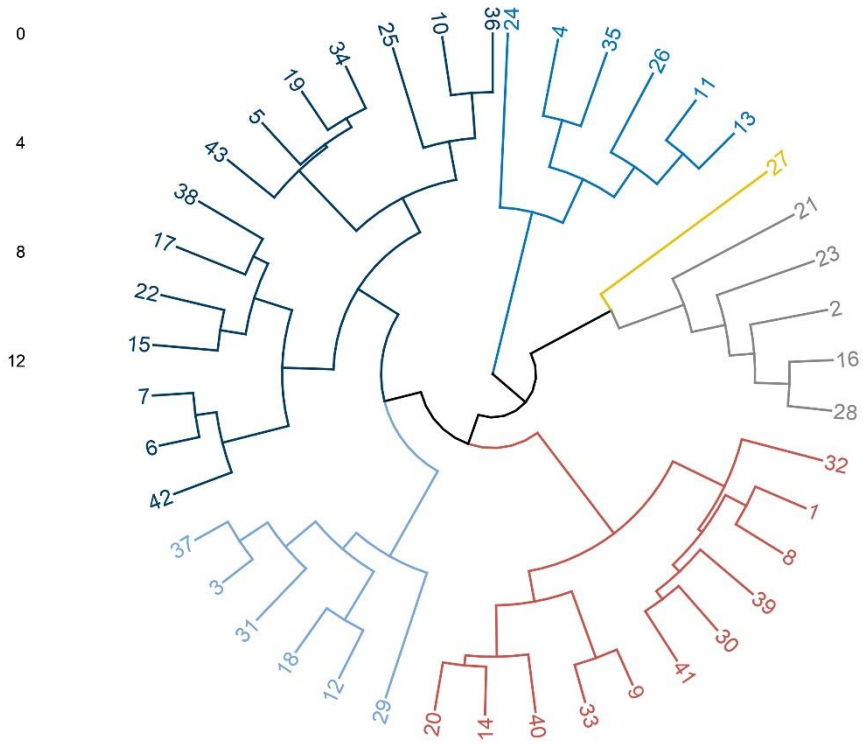

Fig 8: Euclidean distance based Ward D^2clustering of genotypes based on the studied traits.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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