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ASSESSMENT OF GENETIC DIVERSITY IN THE INBRED LINES FOR FORAGE TRAITS IN MAIZE

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ABSTRACT

Maize (*Zea mays* L.) plays a pivotal role in global agriculture, ranking as the third most important cereal crop after wheat and rice. Originating from Mexico, maize has undergone domestication over 7000 years. Teosinte (*Zea mexicana*) is considered the progenitor of cultivated maize. Maize serves dual purposes, providing kernels for human consumption and fodder for cattle feed, making it a crucial cereal forage crop. To address the increasing demand for fodder, optimizing crop productivity through improved varieties and agronomic practices becomes imperative. This study focuses on the assessment of genetic diversity among 50 maize inbred lines, utilizing Mahalanobis D² statistics. Morphological traits, including days to flowering, plant height, leaf characteristics, stem girth, and forage yield, were evaluated. An investigation was carried out to estimate the genetic diversity, combining ability and to assess the relationship between parental diversity and heterosis in newly developed inbred lines for forage traits in maize (*Zea mays* L.) at the College of Agriculture, V. C. Farm, Mandya and Main Research Station, Hebbal during 2018-19. Fifty inbred lines were grouped in to seven clusters using Mahalanobis D² statistic. The cluster II accommodated maximum number of inbred lines (18) followed by cluster III (14). Combining ability analysis was performed using 50 lines ad four testers by employing Line × Tester mating design. The ratio of GCA to SCA variance revealed the preponderance of non-additive gene action in the expression of all the traits under study. The lines viz., 1-50-7, 1-63-5 and 1-17-19 in E 1 ; 1-17-19, 5-6-1 and 1-50-7 in E 2 ; 1-50-7, HCL-7 and 2-4-1-2 on pooled basis and tester CAL-1443 were identified as best general combiners for forage yield and yield related characters. The parents were grouped into four classes based on mean and standard deviation of D² values and found that maximum number of heterotic crosses resulted from parents included in medium divergence classes.

Key words : Genetic diversity, Inbred lines, Heterosis, Maize.

Introduction

Maize (*Zea mays* L.) is the third most important cereal crops, next to wheat and rice in the world agriculture considering both area and productivity. Maize is of American origin, particularly Mexico, having been domesticated about 7000 years ago. Evidences from Botany, Genetics and Cytology have pointed towards a common origin for every existing type of *Zea mays* L. (2n=20). Most researchers believed that the progenitor of cultivated maize is teosinte (*Zea mexicana*) and belongs to family Poaceae and genus *Zea*.

Maize being a dual-purpose crop, the kernels are used for human consumption, while fodder is utilized for cattle feed, thereby maize ranks second position after sorghum among cereal fodder crops. It is almost an ideal cereal forage crop because of its fast growing habit, high palatability and nutritious qualities. Green fodder of maize is ideal for silage making and for utilizing in off-season. It has no toxic compounds and can be fed at any stage of growth. Forage maize has a relatively low cell wall content and high content of non-structural carbohydrates, and as a result, it has high digestibility and bio-energy value.

Green fodder is the cheapest source of feed for milch, beef and draft animals. Therefore, development of fodder resources of the country becomes a high priority national programme. This could be achieved through bringing more area under fodder cultivation and improving productivity of fodder crop coupled with quality. But, there is little scope of increasing area under cultivation of fodder crops due to urbanization, industrialization and traditional inclination among farmers. Only 4.4% of the total cropped area of the country is under fodder crops cultivation. Hence, the only optional strategy to meet fodder requirement is to exploit crop productivity through better yielding varieties and efficient agronomic management.

Information on combining ability effects provides guidelines to the plant breeder in selecting the elite parents and desirable cross combinations to be used in the formulation of systematic breeding programme and at the same time reveals the nature of gene actions involved in the inheritance of various traits. The nature of gene action would help in predicting the effectiveness of selection in a population. A distinct type of gene action, its magnitude and constitution of genetic architecture is of fundamental importance to plant breeder to decide further breeding programme.

Materials and Methods

The details of the material used, methods followed and statistical tools employed for executing the experiment, collection and analysis of the data are presented in this chapter in the following order:

Experimental material and layout

Assessment of genetic diversity in inbred lines

Material for this study included 50 inbred lines of maize developed at AICRP on Forage Crops and Utilization (FCU), ZARS, V. C. Farm, Mandya. These inbred lines were planted in a single row of 3m length with two replications under Randomized Complete Block Design during *kharif*. The spacing followed was 30 cm



Photo 1 : General view of experimental plot at V. C. Farm, Mandya (E₁).



Photo 2 : General view of hybrids and parents evaluation experiment field at Hebbal, Bengaluru (E₂).

between the rows and 15 cm between plants. The data were recorded on morphological characters for diversity analysis.

Characters studied

The observations on green forage yield and its components were recorded on five randomly selected plants for each treatment in each replication and the average value was computed. The procedure adopted for recording each observation is given below.

1. Days to 50 per cent flowering
2. Plant height (cm)
3. Number of leaves per plant
4. Leaf length (cm)
5. Leaf width (cm)
6. Stem girth (cm)
7. Leaf: stem ratio

It was calculated by the following formula:

$$\text{Leaf : stem ratio} = \frac{\text{Fresh weight of all the leaves}}{\text{Fresh weight of stem}}$$

8. Green forage yield per plant (g)
9. Dry matter content (DM %)

$$\text{Dry matter content (DM \%)} = \frac{\text{Oven dry weight}}{\text{Fresh plant weight}} \times 100$$

10. Dry matter yield per plant (g)

It was calculated by using following formula:

$$\text{Dry matter yield per plant} = \frac{\text{Green forage yield per plant}}{100} \times \text{DM\%}$$

Statistical methods

Genetic diversity analysis

Mahalanobis (1936) D^2 statistic was used for assessing the pairwise genetic divergence among test inbred entries. The adjusted mean values were subjected

for phenotypic diversity analysis.

$$D^2_p = d^1 S^{-1} d$$

Where,

D^2_p = Square of distance considering 'p' traits

D = Vector of observed differences of the mean values of 'p' traits

d^1 = Transpose of vector of observed differences of the mean value of 'p' traits

X_i = Vector of the mean values of all the characters

S^{-1} = Inverse of variance and covariance matrix

Since investigating the inverse matrix is complicated, the original correlated variables (X_i) were transformed to non-correlated variables (Y_i). The computation of D^2 values reduced to simple summation of the squares of the difference between the values of transformed variables of the two populations. This transformation was done by pivotal condensation method. These newly transformed uncorrelated variables were used to calculate the square of distance using the formula.

$$D^2_p = (Y_{i1} - Y_{i2})^2$$

Where,

Y = transformed mean values of 'p' traits.

The square root of D^2 provided general distance between two genotypes. D^2 values were arranged in a matrix form. The significance of D^2 values between any two populations was tested using Hotelling's T^2 statistic.

$$T^2 = \frac{(n_1 + n_2) - P - 1}{(n_1 + n_2 - 2)} \times D^2$$

Using T^2 , the F values were calculated

$$F = \frac{(n_1 + n_2) - P - 1}{(n_1 + n_2 - 2)} \times T^2$$

Where,

P = number of traits

n_1 = number of individuals in first population

n_2 = number of individuals in second population

This computed F value was compared with the table F value at five and one percent level of significance at P and $(n_1 + n_2 - P - 1)$ degrees of freedom.

Clustering of genotypes based on the D^2 statistic

The genotypes were grouped into different clusters following Tocher's method as described by Rao (1952). All the calculated $n_2 C_2$ D^2 values were arranged in increasing order of magnitude for each entry. Two genotypes with least distance were considered first and named as cluster I. To this, a third genotype with smallest

distance from the first two genotypes was added. Now the average increase in D^2 value after addition of third genotype to the cluster I was calculated. If this distance was less than the largest D^2 value between any two genotypes in the first row of the table where the D^2 values were arranged in increasing order of magnitude, then the third genotype was included in cluster I. Similarly the possibility of addition of each genotype in cluster I was explored. If the average increase in the D^2 exceeded the threshold, then such genotype was not included in the corresponding cluster and a new cluster was formed. The process was continued until all the genotypes were included into one or the other cluster.

Intra cluster distance

The intra cluster distances were calculated according to Singh and Choudhary (1977).

$$\text{Intra cluster distance} = \sqrt{\frac{D_i^2}{N}}$$

Inter cluster distance

The inter cluster distances were calculated by the formula described by Singh and Choudhary (1977).

$$\text{Inter cluster distance} = \sqrt{\frac{D_i^2}{n_i n_j}}$$

Where,

D_i^2 = sum of squared distances between all possible combinations ($n_i n_j$) of entries included in the clusters i and j .

n_i = number of entries in cluster i

n_j = number of entries in cluster j

Analysis of variance (Simple Lattice Design)

Table 1 : The total variance present in inbreds and hybrids was dissected into the following attributes (Snedecor and Cochran, 1968).

Source of variation	Degrees of freedom	MSS
Replications	$(r-1)$	$RMSS$
Treatments - unadjusted	(t^2-1)	$TMSS$ (unadj.)
- adjusted	(t^2-1)	$TMSS$ (adj.)
Blocks within replications (adjusted)	$r(t-1)$	$BMSS$ (adj.)
Intra-block error	Sub	$EMSS$
Total	(rt^2-1)	

Where,

r = number of replications

t = number of treatments

Analysis of combining ability

Observations recorded on $i \times j^{\text{th}}$ cross grown in j^{th} replication was expressed using a linear model given by Arunachalam (1974).

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + r_k + e_{ijk}$$

Where,

Y_{ij} = value of ij^{th} observation

μ = population mean

g_i = *gca* effect of i^{th} line

g_j = *gca* effect of j^{th} tester

s_{ij} = *sca* effect of ij^{th} cross

r_k = replication effect

e_{ijk} = error associated with $(ijk)^{\text{th}}$ observation

Assessment of general combining ability and specific combining ability

The mean of each character for each entry was subjected to line \times tester analysis and the variance of combining ability was estimated as per the procedure developed by Kempthorne (1957).

Results and Discussion

Evaluation of inbred lines for their morphological and fodder traits

The mean performance of new inbred lines for morphological and fodder traits is presented in Appendix-I and character wise mean and range values are presented in Table 2. The trait days to 50 % flowering ranged from 42 days (1-63-5) to 63 days (1-54-5) among the inbred lines. The mean plant height, stem girth and number of leaves per plant of inbreds varied between 104.20 cm (MAI-715) to 224.00 cm (1-17-19), 6.12 cm (E2-242) to 10.50 cm (1-106-6) and 9 (40013) to 16 (1-104-22), respectively. The average leaf length ranged

from 54.2cm (40013) to 97.88 cm (1-105-3), while the mean leaf width of inbred lines varied between 6.63cm (E₂-176) and 11.68cm (1-106-6). The range for mean of leaf : stem ratio was from 0.17 (1-20-2) to 0.26 (1-17-19) and average green forage yield plant⁻¹ varied between 116.26 g (5-2-1-2) to 514.87 g (5-6-1). The mean dry matter yield plant⁻¹ and dry matter content values ranged from 38.48g (31188) to 198.38g (1-19-5) and 18.36% (2-4-1-2) to 26.68%(1-19-5), respectively.

Genetic diversity analysis

The knowledge of genetic diversity among the genotypes is essential for selecting parents for hybridization programme, especially in a cross-pollinated crop like maize. Genetic diversity considered being an important tool for realizing heterotic response in F₁ and a broad spectrum of variability in segregating generations. Mahalanobi's D^2 statistic is a sensitive tool for assessing genetic divergence for quantitative traits and is widely being used by many geneticists and breeders for selecting divergent parents based on their distances for effecting crosses. By using this genetic distance, it is possible to group the inbreds into different clusters.

Contribution of characters towards total diversity

The contribution of 10 characters towards total morphological diversity is presented in Table 3. Out of 10 characters analyzed, green forage yield plant⁻¹ (73.26%), dry matter yield plant⁻¹ (12.24%) and plant height (9.48%), contributed major proportion to total diversity followed by leaf length (1.58%).stem girth (1.33%), number of leaves plant⁻¹ (1.11%) and leaf : stem ratio (1.00%).

Literature abounds with reports on the usage of rank method of D^2 to get total rank and calculating *per cent* contribution. This method has been criticized for lack of any weightage assigned to different ranks and for the fact that ranking is done on uncorrelated variables (ys)

Table 2 : Character-wise range and mean values of inbreds for forage traits in maize.

S. no.	Character	Range		Mean
		Lowest	Highest	
1	Days to 50 % flowering	1-63-5 (42.00)	1-54-5 (63.00)	49.80
2	Plant height (cm)	MAI-715 (104.2)	1-17-19 (224)	174.27
3	Number of leaves plant ⁻¹	40013 (9.00)	1-104-22 (16.00)	12.45
4	Leaf length (cm)	40013 (54.2)	1-105-3 (97.88)	77.61
5	Leaf width (cm)	E ₂ -176 (6.63)	1-106-6 (11.68)	8.98
6	Stem girth (cm)	E ₂ -242 (6.12)	1-106-6 (10.50)	8.86
7	Leaf - stem ratio	1-20-2 (0.17)	1-17-19 (0.26)	0.19
8	Green forage yield plant ⁻¹ (g)	5-2-1-2 (116.26)	5-6-1 (514.87)	267.73
9	Dry matter yield plant ⁻¹ (g)	31188 (38.48)	1-19-5 (198.38)	115.93
10	Dry matter content (%)	2-4-1-2 (18.36)	1-19-5 (26.68)	20.35

Table 3 : Contribution of different forage characters towards total diversity in maize inbreds.

S. no.	Characters	Rank	Contribution (%)
1	Green forage yield plant ⁻¹ (g)	1	73.26
2	Dry matter yield plant ⁻¹ (g)	2	12.24
3	Plant height (cm)	3	9.48
4	Leaf length(cm)	4	1.58
5	Stem girth(cm)	5	1.33
6	Number of leaves plant ⁻¹	6	1.11
7	Leaf : Stem ratio	7	1.00
8	Days to 50% flowering	8	0.00
9	Leaf width (cm)	10	0.00
10	Dry matter content (%)	11	0.00

Table 4 : Grouping of 50 inbreds based on D^2 statistics and Tocher's method of clustering.

Cluster	No. of genotypes	Cluster members
I	10	3-7-12, E ₂ -94, 1-19-5, 1-17-19, 1-54-5, 40058, 40073, 5-16-1, 1-108-5, 1-63-5
II	18	MAI-767, 40415, 31188, 40013, E ₂ -242, 5-2-1-2, MAI-179, HCL-7, 1-32-3, E ₂ -104, E ₂ -125, MAI-729, 1-17-14, 1-34-2, E ₂ -151, MAI-3, 1-44-9, 1-80-3
III	14	1-50-7, 40104, 1-20-1, 1-65-6, MAI-261, 2-1-32, 4-6-2-2, E ₂ -176, 1-106-6, E ₂ -82, 1-16-2, 5-12-1-1, 1-105-3, MAI-276
IV	5	2-4-1-2, 4-3-2-2, 1-5-12, 5-6-1, 1-20-2
V	1	MAI-224
VI	1	1-104-22
VII	1	MAI-715

which might not be applicable to original correlated means (xs) (Vivek, 2013). Nevertheless, it can be easily determined by coefficient of variation at individual as well as at inter-cluster level (Sharma, 1988). However, both the methods for assessing the contribution of individual characters for total divergence have been discussed. Out of 10 characters, green forage yield per plant has contributed highest for total divergence followed by dry fodder yield per plant and plant height (Table 3). It is evident that the contribution of simply inherited traits which are least affected by extraneous factors was highest for divergence than the characters with complex inheritance and highly affected by extraneous factors (Marker and Krupakar, 2009; Ganesan *et al.*, 2010).

Clustering of inbred lines used for the genetic diversity study

The fifty newly developed inbred lines were grouped into seven clusters based on their genetic distances (Fig. 1 and Table 4). Cluster II was the largest having 18 inbred lines indicating the overall genetic similarity among them and it was followed by cluster III with 14 inbreds, Cluster

I with 10 and cluster IV with five inbred lines. Three were solitary clusters (Cluster V, VI and VII). The grouping of germplasm lines into different clusters indicated the presence of substantial amount of diversity in the material evaluated (Praveena, 2019). Presence of substantial genetic divergence among the genotypes screened in present investigation suggested that this material might serve as good source for selecting the diverse parents for hybridization programme aimed at isolating desirable combination for green forage yield.

Intra and inter cluster distances

Intra and inter cluster distances are presented in Table 5. Highest intra cluster distance was recorded in cluster IV (8724.87) followed by cluster I (4232.29), cluster II

(4163.34) and cluster III (3201.07). The intra cluster distances were lower than the inter-cluster distances. Thus, the genotypes included within a cluster had less diversity among themselves and exhibit a narrow range of genetic variability (Kage *et al.*, 2013).

Highest inter cluster distances were observed between clusters IV and VI (102732.90) followed by cluster II and VI (96341.72), which indicated the wide genetic diversity between these groups. Thus, genotypes with high index for specific characters that fall into different clusters could be intercrossed to achieve maximum hybrid vigour and high frequency of desirable transgressive segregants. Lower estimate of inter cluster distance was noticed between clusters V and VII (6554.20), which indicated the close relationship and likelihood between the genotypes of these clusters. Similar findings were also reported by Jain *et al.* (2006), Bhandari and Verma (2007), Anilkumar *et al.* (2017), Vivek (2013) and Praveena (2019).

The genotypes belonging to the clusters separated by high statistical distance could be used in hybridization

Table 5 : Intra and inter-cluster distances.

Cluster	I	II	III	IV	V	VI	VII
I	4232.29	42220.09	11466.63	19053.16	23740.59	45180.29	22885.57
II		4163.34	14917.83	96341.72	14362.92	9197.47	15437.41
III			3201.07	44570.11	11324.71	17491.80	10433.15
IV				8724.87	61054.38	102732.90	51559.91
V					0.00	31447.86	6554.20
VI						0.00	30147.86
VII							0.00

Table 6 : Cluster means for different quantitative traits in maize inbreds.

Cluster	Days to 50% flowering	Plant height (cm)	No. of leaves per plant	Leaf length (cm)	Leaf width (cm)	Stem girth (cm)	Green forage yield plant ⁻¹ (g)	Dry matter yield plant ⁻¹ (g)	Leaf-stem ratio
I	52.3	202.48	13.16	83.20	8.85	8.66	327.47	159.73	0.19
II	48.61	152.98	11.83	72.36	8.83	8.44	160.63	73.59	0.21
III	48.21	186.41	12.86	79.24	9.06	8.95	252.55	113.88	0.23
IV	49.6	187.48	13.32	82.28	8.60	9.26	442.27	173.73	0.21
V	61.02	113.40	9.80	71.60	9.20	7.10	218.00	156.67	0.22
VI	62.32	220.00	11.20	84.60	8.20	7.40	161.33	36.67	0.26
VII	47.00	104.20	10.60	62.40	9.12	8.24	261.33	92.67	0.27

programme for obtaining a wide spectrum of variation among the segregants. In this context, inbred lines from cluster II, VI and VI should be selected as parents in hybridization programme to produce heterotic forage hybrids in maize. These findings are in conformity with the findings of Ganesan *et al.* (2010).

Hybridization between divergent groups may lead to higher magnitude of heterosis for the characters concerned. However, many earlier studies are of the opinion that crosses between too divergent groups of parents are less successful in achieving required magnitude of heterosis (Arunachalam *et al.*, 1984). On the other hand, the crosses between genotypes exhibiting a narrow range of variability as revealed by lower inter cluster distances may not be worthwhile to get desired extent of heterosis. This is probably because of parents with similarity may possess common alleles governing the characters and may not help in complementation in the hybrid combination. Similarly, parents exhibiting greater divergence may fail to nick well. This is specially being observed in distant crosses (interspecific) for yield related traits. However, many studies have confirmed the fact that parents with moderate divergence exhibits higher frequency of heterotic hybrids (Arunachalam *et al.*, 1984 and Singh *et al.*, 1984).

Cluster mean analysis

Greater range of mean values among the clusters

was recorded for different traits. However, in calculating cluster means, the superiority of a particular genotype with respect to a given character gets diluted by other genotypes that are related and grouped in the same cluster, which are inferior or intermediary for that character under consideration. Hence, apart from selecting genotypes from the clusters, which have high inter-cluster distance for hybridization, one can also think of selecting parents based on extent of genetic divergence with respect to a particular character of interest.

The mean values for different clusters for all the characters were given in Table 6. The cluster VI recorded highest mean value for plant height (220 cm) and leaf length (84.60 cm). Cluster IV recorded highest mean value for number of leaves per plant (13.32), stem girth (9.26 cm) green forage yield plant⁻¹ (442.27 g) and dry matter yield plant⁻¹ (173.73 g). The solitary cluster VI recorded highest mean values for leaf width (9.2 cm), cluster VII recorded highest mean values for leaf : stem ratio (0.27) and lowest for flowering characters.

Cluster IV recorded highest mean values for four traits *viz.*, number of leaves per plant, stem girth, green forage yield per plant and dry matter yield per plant (Table 6). Hence, this cluster could be a candidate for selecting best possible genotypes. The genotypes with high mean values and maximum inter cluster distances could be selected for crop improvement programmes. Similar

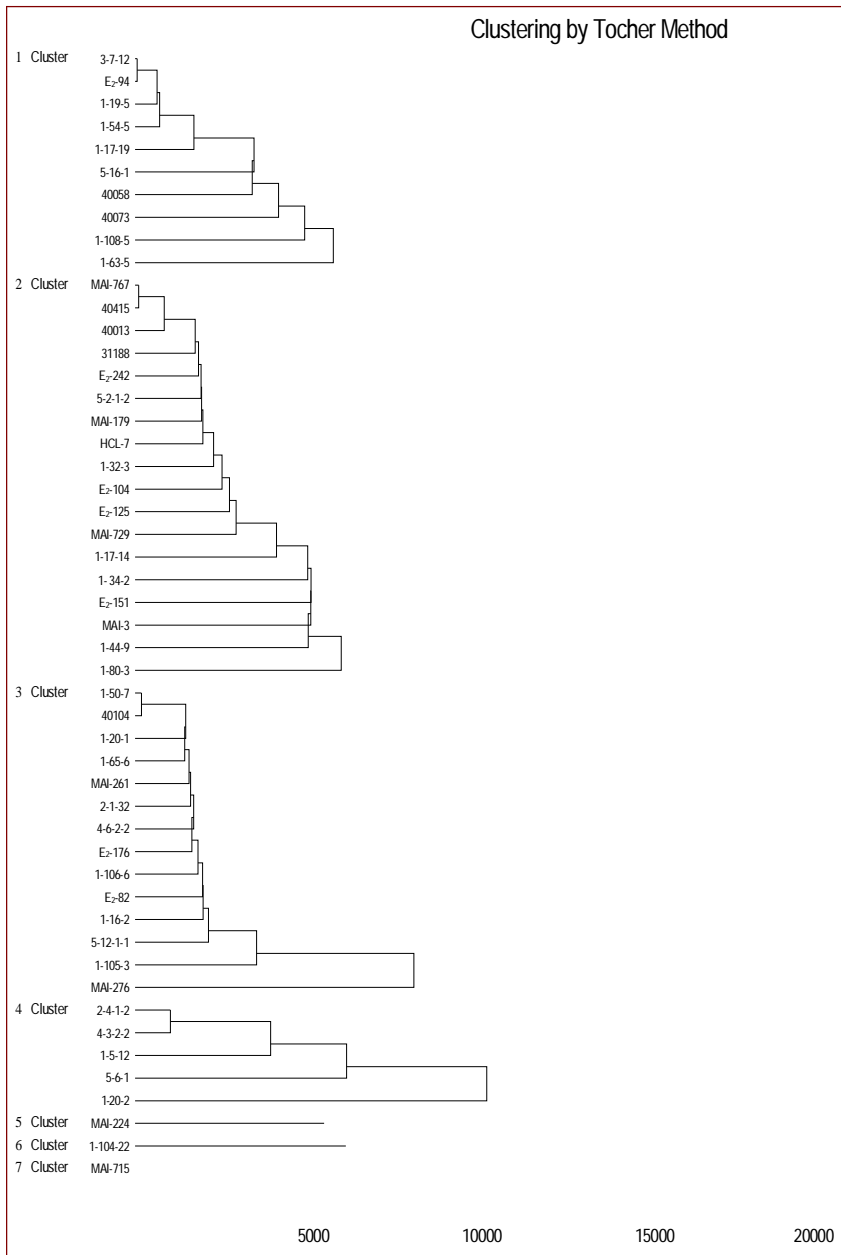


Fig. 1 : Clustering of 50 inbreds based on D^2 statistic and Tocher’s method.

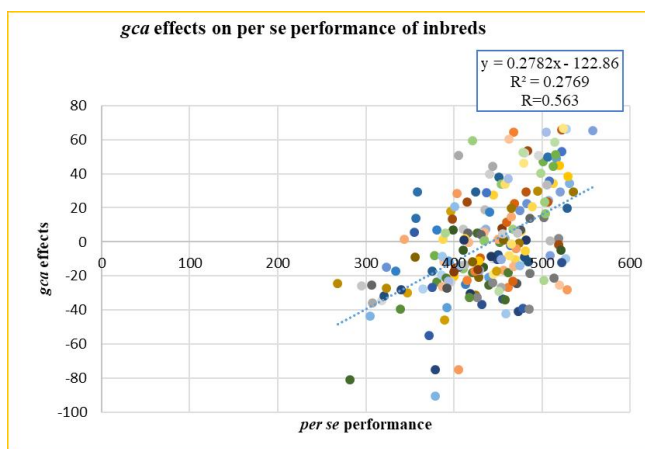


Fig. 2 : *Gca* effects on *per se* performance of inbreds.

results also obtained by Anilkumar *et al.* (2017) and Vivek (2013).

Analysis of combining ability

The actual value of an inbred line for hybrid breeding lies in its worth in hybrid combinations with other inbreds. Visual selection as well as that based on testcross performance during inbreeding helps to eliminate many inbreds, which are poor candidates for use in hybrid development. Even then, a great majority of surviving lines do not end up in highly productive hybrids. The evaluation of inbreds in all possible combinations would be a wastage of resource and call for some shortcuts to eliminate less promising inbreds.

The assessment of combining ability from *per se* performance of inbreds appears to be the simplest approach, but so far has not been reported to be effective in any crop plant and hence, it is not always true that *per se* performance of parent is a good indicator of its potential in hybrid combinations. The concept of combining ability is especially useful in connection with testing procedures, which involve the study and comparison of the performance of homozygous inbreds in hybrid combinations.

Combining ability studies provide information on the genetic mechanisms controlling the inheritance of quantitative traits and enable the breeders to select suitable parents for further improvement or use in hybrid breeding for commercial purposes. Line \times Tester mating design was developed by Kempthorne (1957), which

provides reliable information on the general and specific combining ability effects of parents and their hybrid combinations.

Conclusion

For the assessment of genetic diversity, 54 inbred lines were evaluated for forage traits during *kharif* 2018 at Zonal Agriculture Research Station, V.C. Farm, Mandya, using Mahalanobi’s D^2 analysis. Results revealed seven distinct clusters which indicated that the material had genetic variation. The highest inter-cluster distance was observed between cluster IV (five genotypes) and cluster VI (single genotype) and the

lowest between the cluster V (single genotype) and VI (five genotypes). Clustering pattern revealed that genetic diversity depends on contribution of forage traits like plant height, stem girth, number of leaves per plant, leaf length, green forage yield and dry fodder yield per plant. Highly significant differences in mean sum of squares due to parents for green forage yield and its component traits were observed that justified the selection of parents for the study.

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