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Research Article



Genetic distance as a predictor of heterosis in single cross hybrids of fodder maize (*Zea mays* L.)

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Abstract

Assessment of genetic distance between parents and selection of diverse parents are extensively used in many crops. Current study focuses the importance of genetic distance assessed through SSR markers (GD_{MOL}) and morphological traits (GD_{MOR}) in 28 fodder maize inbred lines and its impact on heterotic expression in resultant 195 single cross fodder maize hybrids. While comparing the mean green fodder yield in top performing hybrids synthesised with parents of higher GD_{MOL} and GD_{MOR} , it is understood that the genetic distance had definite impact on yielding heterotic hybrids with superior green fodder yield. It is evident from identification of a single cross UMI 1200 X GETM 25 with parents of greater GD_{MOL} and GD_{MOR} and a cross N 66 X DM 12-4 with parents of higher GD_{MOR} recorded greater green fodder yield and superior standard heterosis (20.88%). The correlation studies revealed significant positive association between higher GD_{MOL} of parents and heterotic performance of hybrids for crude protein and desirable negative association with crude fibre, ADF and NDF.

Keywords: Fodder maize, genetic distance, hybrids, SSR markers

INTRODUCTION

Maize is one of the most versatile crops, provides food, feed, and raw materials for industrial use. Traditionally, maize is grown primarily as a subsistence crop to meet food and fodder needs. Being a cross pollinated crop, crop breeders experienced success in exploitation of heterosis to improve the grain as well as fodder yield in this crop. The prediction of heterosis was challenging due to limitations in conventional techniques based on genetic relationship, place of origin, phenotypic markers and isozymes (Zhu *et al.*, 1987; Hinze and Lamkey, 2003). The discovery of molecular marker techniques offers a novel approach to estimate heterosis, significantly enhancing the accuracy of hybrid breeding.

Maize breeders are widely aware that greater heterotic expression in hybrids can be produced by greater diversity among parental inbreds (Moll *et al.*, 1965; Hallauer, 1972). To solve challenges in improving heterosis and to produce diverse inbred lines, utilisation of molecular markers based genetic distances estimation in parents has been recommended (Ndhlela *et al.*, 2015). This has the advantage of selectively allowing suitable heterotic crosses between the farthest inbreds.

DNA markers have evolved into practical tools for identifying genetic variation and projecting heterosis in various crops. In Rice (Zhang *et al.*, 1996), Maize (Betran

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et al., 2003; Kiula et al., 2008), Oilseed (Riaz et al., 2001), and Wheat (Lee et al., 1995), there have been reports of positive relationships between genetic distance (GD) and heterosis. However, some studies also revealed a lack of correlation or a weak correlation between GD and heterosis in Maize (Melchinger et al., 1990), Rice (Saghai Maroof et al., 1997). Also, in cotton, the studies have established the relationship between the genetic distance of crosses and appearance of superior segregants in F₂ and F₃ populations (Ganesan et al., 2005). Numerous investigations have been published about genetic diversity of parental genotypes and their relationship on heterotic performance of hybrids. Moll et al. (1962) used geographic origin as a criterion to evaluate GD between parental genotypes in maize. They discovered that heterosis increased linearly with the extension of GD (Moll et al., 1962), but decreased among those exceedingly wide crossings (Moll et al., 1965). Any crop improvement programme typically requires genetic diversity as it aids in the creation of better hybrids (Naik et al., 2006).

The effectiveness of a breeding programme essentially depends on the direction and strength of the relationship between the yield and its constituent parts as well as the proportionate weight each element has on forage yield. Correlation analysis assesses the strength and direction (positive or negative) of the link between two or more variables. The roles and relative contributions of different component traits on expression of yield under specific environment would very well be understood through correlation analysis (Shahbaz *et al.*, 2007).

Therefore, it is necessary to correlate the yield and genetic divergence of several numbers of inbred lines that have been gathered from various sources. So, this study was carried out to relate the higher genetic distance between the parents and their heterotic performance in resultant hybrids.

MATERIALS AND METHODS

Plant genetic material: A total of 28 fodder maize inbreds maintained in the Department of Forage Crops, Tamil Nadu Agricultural University, Coimbatore were used in this study. The inbred lines such as UMI 61, UMI 112, UMI 1200, UMI 1201, UMI 1205, N-09-160-2, N-10-86, N 66, 52021, 52485, DM 82, DM 84 and GETM 14 were used as lines whereas inbreds *viz.*, DM 12, DM 12-1, DM 12-4, DM 12-5, DM 12-6, DM 74-2, UMI 1210, UMI 1221, GETM 25, GETM 26, GETM 39, GETM 40, GETM 67, TNFM 139-1 and African tall were used as testers.

Assessment of genetic distance : The inbred lines were raised during *kharif* 2021 and *rabi* 2022 for evaluating the morphological and forage quality traits. Based on 12 biometrical and four quality parameters, the pairwise genetic distance (GD_{MOR}) was calculated. All these genotypes were also genotyped using 30 SSR markers and genetic distance (GD_{MOL}) at a molecular level was estimated.

Development of hybrids and evaluation: Hybridization was performed with aforesaid 13 inbreds as lines and 15 inbreds as testers in a L x T fashion during *Summer* 2022. A total of 195 single cross hybrids were developed and evaluated for two seasons during *kharif* 2022 and *rabi* 2022 for their *per se* as well as heterotic performance. The hybrids were evaluated in Randomized Complete Block Design with two replications with a spacing of 30 cm x 15 cm. All the hybrids were maintained with similar agronomic management and evaluated for biometrical and forage quality traits. The biochemical analysis *viz.,* crude protein, crude fiber, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were recorded with the help of Near Infrared Spectrophotometer (NIR) (Model Spectra Alyser, ZEUTECH, Germany).

Statistical analysis: The pairwise parental line genetic distance was estimated in DARwin (version 6.0.21) software (Perrier & Jacquemoud-Collet, 2006) for both morphological (GD_{MOR}) and molecular (GD_{MOL}) basis. The $\mathrm{GD}_{_{\mathrm{MOR}}}$ and $\mathrm{GD}_{_{\mathrm{MOL}}}$ were calculated by using Euclidean diversity coefficient distance (Champely and Chessel, 2002) and Dice coefficient (Dice, 1945) respectively with 1000 time's bootstrap method. The GD_{MOR} of parents was analysed by using the parameters viz., days to 50 per cent flowering, plant height, cob height, leaf length, leaf breadth, number of leaves, number of nodes, stem girth, internode length, leaf stem ratio, crude protein, crude fiber, acid detergent fiber, neutral detergent fiber, dry matter yield and green fodder yield. Whereas, the $\mathrm{GD}_{\mathrm{MOL}}$ of parents was measured by using 110 alleles of 30 SSR markers. The heterosis values of all the hybrids were calculated with the help of TNAUSTAT (Manivannan, 2014). The correlation analysis between traits and heterosis values was analysed using R packages viz., 'variability' and the matrix was visualized using package 'ggcorrplot'. The association analysis of traits for two seasons was analysed using R packages.

Relationship of the parental genetic distance and their heterotic performance in hybrids: The pair wise genetic distance between 28 fodder maize inbred lines based on molecular diversity (GD_{MOL}) and morphological diversity (GD_{MOR}) were presented in **Table 1**. The association between genetic distance based on SSR markers (GD_{MOL}) and morphological diversity (GD_{MOL}) and morphological diversity (GD_{MOR}) of parents on heterotic performance in hybrids is furnished in the **Table 2**.

Association between genetic distance of parents and mid parental heterosis: The correlation between the GD_{MOL} of parents and mid parental heterosis of their hybrids revealed the presence of a positive significant association for crude protein. This positive association for important quality trait crude protein was observed during both *kharif* 2022 (0.244) and *rabi* 2022 (0.157) seasons. Whereas, a significant negative association was observed with the traits like crude fiber, ADF and NDF in *kharif* 2022. The significant correlation of heterosis with SSR based genetic

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25	4.33 4	4.68 4	5.01 4	4.50 4	5.62 4	3.56	5.12	4.48 4	5.07 3	3.69 3	4.76 4	3.63	3.24 3	5.47 5	5.14 4		6.29 (6.29 (6.32 (6.29 6 6.32 6 6.10 6	6.29 6 6.32 6 6.10 6 6.77 1	6.29 6 6.32 6 6.10 6 6.77 7 6.55 6	6.29 6 6.32 6 6.10 6 6.77 7 6.77 7 6.55 6	6.29 6 6.32 6 6.10 6 6.77 7 6.55 6 6.55 6 6.17 7 7.36 4	6.29 6 6.32 6 6.10 6 6.77 7 6.77 7 6.55 6 6.17 6 6.17 4 1.36 4 4.50 4	6.29 6 6.32 6 6.10 6 6.17 7 6.77 7 6.55 6 6.17 6 6.17 6 7.36 6 4.50 4 4.50 4	6.29 6 6.32 6 6.10 6 6.17 7 6.17 7 6.55 6 6.17 6 6.17 9 4.50 4 4.38 7 4.38 7 4.38 7 4.38 7 4.38 7 6.55 6	6.29 6 6.32 6 6.10 6 6.17 7 6.17 6 6.17 6 6.17 9 4.50 4 4.50 4 4.50 2 0.33	6.29 6 6.32 6 6.10 6 6.17 7 6.17 6 6.17 6 6.17 7 7.36 6 6.17 7 4.53 7 4.53 7 .36 0 .33 0 .33 0 0.33 0
24	4.80	6.72	6.53	6.22	7.19	5.10	5.62	6.86	5.91	5.71	6.34	6.63	4.56	4.93	5.92		6.85	6.85 6.81	6.85 6.81 5.74	6.85 6.81 5.74 6.99	6.85 6.81 5.74 6.99 6.51	6.85 6.81 5.74 6.99 6.51 7.63	6.85 6.81 5.74 6.99 6.51 7.63 7.69	6.85 6.81 5.74 6.99 6.51 7.63 7.63 6.47	6.85 6.81 5.74 6.99 6.51 7.63 7.69 6.47	6.85 6.81 5.74 6.99 6.51 7.63 6.47 6.47 0.45	6.85 6.81 5.74 6.99 6.51 7.63 6.47 6.47 0.45 0.47	6.85 6.81 5.74 6.99 6.51 7.69 6.47 6.47 0.45 0.47
23	5.25	5.24	5.28	3.36	4.61	4.01	7.34	4.36	4.71	3.87	3.81	3.94	4.75	5.75	3.75		5.20	5.20 5.46	5.20 5.46 5.36	5.20 5.46 5.36 5.82	5.20 5.46 5.36 5.82 6.49	5.20 5.46 5.36 5.82 6.49 3.81	5.20 5.46 5.36 5.36 5.82 6.49 3.81 5.84	5.20 5.46 5.36 5.82 6.49 3.81 5.84	5.20 5.46 5.36 5.82 6.49 6.49 5.84 5.84 0.41	5.20 5.46 5.36 5.32 6.49 3.81 5.84 5.84 5.84 0.41	5.20 5.46 5.36 5.82 6.49 6.49 3.81 5.84 5.84 0.45 0.45	5.20 5.46 5.35 5.35 6.49 6.49 3.81 5.84 5.84 0.41 0.45 0.37
22	5.60	8.69	7.58	5.35	5.70	6.85	10.45	5.14	8.31	7.44	6.11	7.58	7.33	6.46	6.07		6.40	6.40 6.31	6.40 6.31 6.18	6.40 6.31 6.18 7.44	6.40 6.31 6.18 7.44 4.94	6.40 6.31 6.18 7.44 4.94 4.94	6.40 6.31 6.18 7.44 4.94 4.94	6.40 6.31 6.18 7.44 4.94 4.56 4.56 0.46	6.40 6.31 6.18 7.44 4.94 4.56 0.46 0.43	6.40 6.31 6.18 6.18 7.44 4.94 4.56 0.46 0.46	6.40 6.31 6.18 7.44 4.94 4.56 0.46 0.46 0.43 0.43	6.40 6.31 6.18 7.44 4.94 4.56 4.56 0.46 0.43 0.43 0.51 0.50
21	4.95	6.97	6.84	4.32	4.68	6.09	9.53	4.93	7.46	6.30	5.47	6.49	6.50	5.05	3.54		3.41	3.41 3.90	3.41 3.90 4.06	3.41 3.90 4.06 4.55	3.41 3.90 4.06 4.55 4.55	3.41 3.90 4.06 4.55 4.55	3.41 3.90 4.06 4.55 4.50 4.50 0.37	3.41 3.90 4.06 4.55 4.50 4.50 0.37 0.37	3.41 3.90 4.06 4.55 4.50 0.37 0.37 0.38	3.41 3.90 4.55 4.55 4.55 4.50 0.37 0.37 0.37 0.38	3.41 3.90 4.55 4.55 4.50 4.50 0.37 0.37 0.37 0.38 0.38	3.41 3.90 4.55 4.55 4.50 4.50 0.37 0.37 0.38 0.38 0.38 0.38
20	4.39	8.06	8.08	5.79	5.03	6.49	9.63	6.48	8.22	7.16	7.25	7.88	6.78	4.09	4.45		3.93	3.93 4.02	3.93 4.02 3.55	3.93 4.02 3.55 5.06	3.93 4.02 3.55 5.06	3.93 4.02 3.55 5.06 0.29	3.93 4.02 3.55 5.06 5.06 0.29 0.29	3.93 4.02 3.55 5.06 5.06 0.29 0.47 0.51	3.93 4.02 5.06 5.06 0.29 0.47 0.51	3.93 4.02 3.55 5.06 5.06 0.29 0.29 0.47 0.51 0.51	3.93 4.02 5.06 5.06 0.29 0.29 0.47 0.51 0.51 0.51 0.38 0.38	3.93 4.02 5.06 5.06 0.29 0.47 0.29 0.47 0.47 0.40 0.43 0.49
19	5.50	7.35	7.76	5.80	5.83	6.89	9.00	7.19	7.98	7.35	7.38	7.91	6.87	4.13	3.39	000	3.29	3.29 3.77	3.29 3.77 2.57	3.29 3.77 2.57	3.29 3.77 2.57 0.49	3.29 3.77 2.57 0.49 0.42	3.29 3.77 2.57 2.57 0.49 0.49	3.29 3.77 2.57 2.57 0.49 0.49 0.49 0.43	3.29 3.77 2.57 2.57 0.49 0.49 0.53 0.53	3.29 3.77 2.57 2.57 0.49 0.49 0.53 0.53 0.53	3.29 3.77 2.57 2.57 0.49 0.49 0.53 0.53 0.55 0.55	3.29 3.77 2.57 2.57 0.49 0.49 0.53 0.53 0.53 0.53 0.53 0.55 0.55
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13	4.32	3.51	3.69	3.85	4.91	2.66	3.88	4.58	3.85	3.82	3.99	4.27		0.59	0.67	77 U	1.0	0.65	0.58	0.65 0.58 0.58 0.53	0.65 0.58 0.53 0.53 0.67	0.65 0.58 0.53 0.53 0.67 0.67	0.65 0.65 0.53 0.53 0.67 0.67 0.67	0.65 0.58 0.58 0.53 0.67 0.67 0.67 0.74 0.67	0.65 0.65 0.58 0.53 0.67 0.67 0.67 0.74 0.74 0.55	0.65 0.58 0.53 0.53 0.67 0.67 0.67 0.67 0.67 0.65 0.65	0.65 0.65 0.53 0.67 0.67 0.67 0.67 0.67 0.67 0.65 0.65 0.69	0.65 0.658 0.588 0.53 0.67 0.67 0.67 0.67 0.67 0.55 0.55 0.76 0.57
12	6.45	5.49	5.14	3.82	5.15	3.22	6.06	4.64	4.10	3.00	3.64		0.49	0.53	0.39	0.53		0.48	0.48 0.51	0.48 0.51 0.53	0.48 0.51 0.53 0.66	0.48 0.51 0.53 0.66 0.66	0.48 0.51 0.53 0.66 0.66 0.78 0.78	0.48 0.51 0.53 0.66 0.78 0.68 0.68	0.48 0.51 0.53 0.66 0.78 0.78 0.68 0.68	0.48 0.51 0.53 0.66 0.78 0.68 0.68 0.64 0.61 0.61	0.48 0.51 0.53 0.53 0.66 0.78 0.64 0.64 0.61 0.67	0.48 0.51 0.53 0.66 0.68 0.68 0.64 0.61 0.61 0.67
7	5.66	4.91	4.00	2.77	4.62	3.74	6.20	3.78	4.03	4.47		0.34	0.56	0.54	0.54	0.66		0.60	0.60 0.59	0.60 0.59 0.59	0.60 0.59 0.59 0.76	0.60 0.59 0.59 0.76 0.65	0.60 0.59 0.59 0.76 0.65 0.65	0.60 0.59 0.59 0.76 0.65 0.65 0.61	0.60 0.59 0.59 0.76 0.65 0.61 0.61 0.59	0.60 0.59 0.76 0.76 0.65 0.65 0.61 0.59 0.59 0.62	0.60 0.59 0.59 0.76 0.65 0.61 0.61 0.59 0.59 0.59 0.70	0.60 0.59 0.59 0.76 0.65 0.65 0.61 0.62 0.61 0.70 0.61
10	5.95	5.29	5.35	4.61	5.47	2.98	5.99	5.42	3.32		0.47	0.41	0.49	0.49	0.44	0.56		0.53	0.53 0.50	0.53 0.50 0.55	0.53 0.50 0.55 0.58	0.53 0.50 0.55 0.58 0.63	0.53 0.50 0.55 0.58 0.63 0.67	0.53 0.50 0.55 0.58 0.63 0.67 0.67	0.53 0.50 0.55 0.58 0.53 0.67 0.67 0.67 0.55	0.53 0.55 0.55 0.58 0.63 0.67 0.61 0.61 0.55	0.53 0.55 0.55 0.58 0.58 0.67 0.67 0.61 0.55 0.55 0.59	0.53 0.50 0.55 0.58 0.63 0.67 0.67 0.61 0.55 0.55 0.59 0.48
6	6.79	4.94	5.32	4.89	5.71	3.70	4.96	6.28		0.41	0.48	0.36	0.43	0.59	0.44	0.57		0.48	0.48 0.51	0.48 0.51 0.59	0.48 0.51 0.59 0.67	0.48 0.51 0.59 0.67 0.67 0.62	0.48 0.51 0.59 0.67 0.62 0.66	0.48 0.51 0.59 0.67 0.67 0.66 0.66	0.48 0.51 0.59 0.67 0.67 0.66 0.74 0.74 0.74	0.48 0.51 0.59 0.67 0.67 0.66 0.66 0.74 0.74 0.59 0.62	0.48 0.51 0.59 0.67 0.62 0.66 0.74 0.74 0.59 0.62 0.60	0.548 0.51 0.59 0.67 0.67 0.66 0.74 0.59 0.59 0.59 0.60
8	3.98	5.15	3.87	3.41	4.12	4.60	7.13		0.34	0.34	0.61	0.46	0.51	0.67	0.46	0.58	L	0.CO	0.49 0.49	0.57 0.57	0.55 0.57 0.55	0.49 0.49 0.57 0.55 0.60	0.65 0.57 0.55 0.55 0.60 0.60	0.65 0.49 0.57 0.55 0.55 0.60 0.67 0.63	0.67 0.49 0.57 0.55 0.55 0.60 0.67 0.63 0.63	0.49 0.49 0.55 0.65 0.60 0.67 0.63 0.63 0.63 0.68	0.49 0.49 0.55 0.55 0.67 0.67 0.67 0.63 0.63 0.63 0.63	0.49 0.49 0.57 0.55 0.65 0.67 0.63 0.63 0.63 0.63 0.63 0.63
7	6.67	5.08	5.55	6.54	7.66	5.05		0.31	0.41	0.49	0.51	0.50	0.52	0.56	0.41	0.59		0.57	0.57	0.57 0.51 0.53	0.57 0.53 0.53	0.51 0.53 0.53 0.59 0.50	0.51 0.53 0.53 0.59 0.50	0.51 0.53 0.53 0.59 0.50 0.50	0.57 0.53 0.53 0.50 0.50 0.50 0.50 0.50 0.71	0.51 0.53 0.53 0.53 0.50 0.50 0.60 0.71 0.71	0.51 0.53 0.53 0.59 0.50 0.50 0.50 0.71 0.71 0.71 0.59	0.51 0.53 0.53 0.50 0.50 0.60 0.60 0.71 0.71 0.71 0.63 0.63 0.63 0.63
9	9.4.81	4.44	5 4.41	3.27	4.22	_	0.46	0.45	0.46	0.51	0.53	0.49	0.57	0.61	0.55	3 0.58		0.56	0.56 0.58 0.58	0.56 0.58 0.61	0.56 0.58 0.61 0.66	0.56 0.58 0.61 0.61 0.66 0.66	0.56 0.58 0.61 0.66 0.66 0.63 0.63	0.56 0.58 0.61 0.66 0.66 0.63 0.63	0.56 0.58 0.61 0.63 0.63 0.63 0.63 0.63 0.67 0.67 0.63	0.56 0.58 0.61 0.63 0.66 0.63 0.67 0.67 0.67 0.63 0.63 0.58	0.56 0.61 0.61 0.61 0.66 0.67 0.63 0.63 0.63 0.63 0.63 0.63 0.63 0.63	 0.56 0.58 0.61 0.66 0.63 0.63 0.63 0.63 0.67 0.67 0.67 0.67 0.58 0.58 0.58 0.62 0.67
5	9.4.65	3 5.31	3 5.05	3.16	6	2 0.51	3 0.36	2 0.35	7 0.45	1 0.41	3 0.55	5 0.54	9 0.51	3 0.52	0.47	7 0.63		30.068	3 0.68 1 0.63	3 0.68 1 0.63 1 0.63	 > 0.68 1 0.63 1 0.63 4 0.65 	> 0.68 1 0.63 1 0.63 2 0.65 8 0.65	> 0.68 1 0.63 1 0.63 2 0.65 0.65 0.65 0.65	 ○ 0.68 1 0.63 1 0.63 3 0.66 1 0.65 1 0.65 1 0.65 	 0.68 0.63 0.65 0.65 0.66 0.66 0.66 0.66 0.66 0.66 0.66 0.66 	 0.68 0.63 0.65 0.66 0.66 0.66 0.66 0.66 1 0.66 	 0.68 0.63 0.66 	0.68 0.63 0.63 0.66 0.66 0.66 0.66 0.66 0.66
4	3 4.60	9 5.16	4.33	£	3 0.06	1 0.52	3 0.36	3 0.32	5 0.47	4 0.44	4 0.53	3 0.55	5 0.45	3 0.56	3 0.50	2 0.67		2 0.73	2 0.73 3 0.6 [′]	2 0.73 5 0.61 4 0.6 [′]	2 0.73 5 0.61 4 0.61 3 0.62	2 0.73 5 0.61 4 0.61 3 0.62 3 0.62	2 0.73 5 0.61 4 0.61 3 0.64 0 0.55 3 0.65	2 0.73 5 0.61 4 0.61 3 0.64 3 0.64 3 0.55 5 0.6 ²	2 0.73 5 0.61 4 0.61 7 0.62 7 0.55 8 0.65 5 0.67 5 0.67	2 0.73 5 0.61 4 0.61 3 0.64 3 0.64 5 0.67 5 0.67 5 0.67 2 0.67	2 0.73 0.61 1 0.61 1 0.61 1 0.61 2 0.67 2 0.67 2 0.67 2 0.67 2 0.67 2 0.67 3 0.67 5 0.77 5 0.77	2 0.73 0.61 1 0.61 2 0.61 2 0.67 5 0.67 5 0.67 5 0.67 2 0.67 2 0.67 3 0.57 2 0.67 3 0.57 5 0.67 5 0.67 5 0.57 5 00
33	9 5.06	3.36	2	7 0.26	2 0.33	7 0.61	3 0.35	5 0.26	9 0.35	4 0.44	9 0.54	3 0.53	5 0.55	4 0.66	1 0.53	3 0.62		0.62 C	0.62 0.56	0.62 4 0.56 9 0.6 ²	0.62 4 0.56 9 0.64 8 0.55) 0.62 4 0.56 9 0.64 8 0.55) 0.62 9 0.64 8 0.55 9 0.55 3 0.65) 0.62 9 0.64 9 0.65 9 0.55 3 0.55 8 0.65	 0.62 0.56 0.64 0.64 0.65 0.55 0.55 0.55 0.55 0.55 	 0.62 4.0.56 4.0.56 3.0.62 3.0.65 3.0.65 5.0.56 5.0.56 7.0.62) 0.62 9 0.64 9 0.66 3 0.55 9 0.55 6 0.56 7 0.65 7 0.65 3 0.66	0.062 0.062 0.062 0.064 0.064 0.055 0.056 0.066 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067 0.067
7	5.26	8	4 0.25	0.37	2 0.42	4 0.47	3 0.48	3 0.35	0.35	3 0.44	3 0.45	3 0.48	3 0.65	3 0.54	5 0.5	5 0.63		1 0.6(1 0.60 1 0.52	1 0.60 1 0.54 4 0.55	1 0.60 1 0.5∠ 4 0.55 4 0.65	1 0.60 1 0.54 4 0.55 4 0.65 1 0.55	1 0.60 1 0.54 4 0.55 1 0.55 1 0.55 1 0.65	1 0.60 1 0.54 4 0.55 1 0.65 1 0.65 8 0.65	1 0.60 1 0.54 4 0.55 4 0.56 1 0.56 8 0.66 5 0.64	1 0.60 1 0.54 1 0.55 1 0.55 1 0.55 8 0.65 8 0.65 8 0.55	1 0.56 1 0.57 1 0.57 1 0.57 8 0.65 8 0.65 8 0.65 8 0.57 4 0.57	1 0.56 1 0.57 1 0.57 1 0.57 8 0.65 8 0.66 8 0.57 1 0.56
-		0.35	0.34	0.30	0.32	0.54	0.46	0.36	0.40	0.46	0.56	0.46	0.46	0.68	0.55	0.65	5	0.7	0.71	0.71 0.6 [°]	0.71 0.64 0.62 0.62	0.6 ² 0.6 ² 0.6 ² 0.6 ²	0.61 0.62 0.62 0.62 0.67	0.67 0.66/ 0.66/ 0.66/ 0.67/ 0.57	0.71 0.62 0.62 0.62 0.72 0.55	0.51 0.61 0.62 0.62 0.55 0.55 0.55	0.61 0.64 0.62 0.67 0.55 0.55 0.55 0.55 0.55	0.07 0.06 0.07 0.05 0.55 0.55 0.55
	~	2	с	4	5	9	7	8	6	10	5	12	13	14	15	16		17	17 18	17 18 19	17 18 19 20	17 18 19 20 21	17 19 20 21 22	17 19 20 21 22 23 23	17 19 21 22 23 23 24 24	17 19 20 21 22 23 24 23 25 25	17 19 21 22 23 25 25 26 26	17 19 21 22 23 25 25 25 25 27 27

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Traits	I	Mid paren	t heterosi	s	В	etter pare	nt heteros	sis	Standard heterosis					
	Khar	if2022	Rabi	2022	Khar	if2022	Rabi	2022	Khar	if2022	Rabi 2022			
	GD _{MOL}	GD _{MOR}	GD _{MOL}	GD _{MOR}	GD _{MOL}	GD _{MOR}	GD _{MOL}	GD _{MOR}	GD _{MOL}	GD _{MOR}	GD _{MOL}	GD _{MOR}		
DFF	-0.098	0.136	-0.142	0.031	-0.073	0.034	-0.090	-0.073	-0.066	0.346	-0.108	0.273		
СН	0.025	-0.183	0.010	-0.015	0.022	-0.404	-0.001	-0.176	0.156	-0.046	0.134	0.155		
PH	-0.076	-0.226	-0.129	-0.148	-0.073	-0.582	-0.160	-0.307	0.066	-0.035	0.086	0.072		
NL	-0.102	0.228	0.089	-0.017	-0.192	0.085	0.013	-0.110	0.023	0.083	0.157	0.079		
NN	-0.037	0.094	0.060	-0.034	-0.113	-0.089	-0.008	-0.103	-0.008	0.013	0.145	0.032		
LL	-0.166	-0.097	-0.038	0.008	-0.189	-0.299	-0.102	-0.049	0.012	0.006	-0.004	0.090		
LB	-0.027	0.118	0.015	-0.072	-0.014	0.124	0.030	-0.024	-0.010	0.047	-0.046	0.065		
IL	-0.161	-0.331	-0.194	0.060	-0.173	-0.409	-0.233	0.063	0.012	-0.239	0.036	0.039		
SG	-0.027	0.028	-0.033	-0.114	-0.069	-0.123	-0.141	-0.169	0.046	-0.025	0.044	0.070		
LSR	-0.033	0.250	-0.056	0.092	-0.022	0.238	-0.011	0.090	-0.055	0.018	-0.150	-0.106		
CP	0.244	-0.106	0.157	-0.135	0.244	-0.187	0.140	-0.209	0.152	-0.218	0.050	-0.196		
CF	-0.212	-0.101	-0.128	0.061	-0.245	-0.059	-0.132	0.057	-0.072	-0.059	-0.059	0.085		
ADF	-0.164	-0.093	-0.103	0.073	-0.169	-0.170	-0.117	0.051	-0.041	0.038	-0.077	0.116		
NDF	-0.256	0.146	-0.016	-0.086	-0.257	0.159	-0.009	-0.129	-0.079	0.062	-0.101	0.062		
DMY	-0.076	0.117	-0.013	-0.191	-0.060	0.100	0.025	-0.161	-0.134	0.151	0.049	0.071		
GFY	-0.047	-0.039	0.047	-0.211	-0.062	-0.207	0.102	-0.229	-0.087	0.081	0.038	0.136		

Table 2. Correlation between genetic distance and heterosis performance during Kharif 2022 and Rabi 2022

Bold values- significance @5%.

GD_{MOL}- Genetic distance based molecular diversity, GD_{MOR}- Genetic distance based morphological diversity, DFF-days to fifty per cent flowering, PH- plant height, CH- Cob placement height, LL- Leaf length, LB –Leaf breadth, NL- Number of leaves, NN- Number of nodes, SG- Stem girth, IL- Internode length, LSR- Leaf stem ratio, CP- Crude protein, CF- Crude fiber, ADF- Acid detergent fiber, NDF- Neutral detergent fiber, DMY- Dry matter yield, GFY- Green fodder yield.

distance indicated the potential usefulness of molecular markers to estimate the hybrid performance with improved quality. Similar prediction of heterosis performance using AFLP markers based on genetic distance in maize was reported by Kilua *et al.* (2008) and Legesse *et al.* (2008).

The association based on the GD_{MOR} of parental genotypes with mid parent heterosis showed a positive relationship for the number of leaves (0.228), leaf stem ratio (0.250) and NDF (0.146) in the *kharif* 2022. However during *rabi* 2022, yield traits namely green fodder yield (-0.211) and dry matter yield (-0.191) showed a significant negative correlation with mid parental heterosis.

Wegary *et al.* (2013) also studied the positive relationship between maize grain yield with mid parent heterosis. However, they reported inconsistency in the association of traits with GD_{MOL} and GD_{MOR} of parents at different locations. Chen *et al.* (2010) and Pandey *et al.* (2018) also reported that parental GD_{MOL} affects the heterotic expressions in hybrids.

Association between genetic distance of parents and better parental heterosis: The correlation between better parent heterosis and GD_{MOL} of parental inbreds displayed the presence of significant negative associations

with the traits number of leaves (-0.192), leaf length (-0.189), internode length (-0.173), crude fibre (-0.245), ADF (-0.169) and NDF (-0.257) in *kharif* 2022. Among these, the traits like leaf length, internode length and ADF had the same negative association with GD_{MOR} of corresponding parents in the *kharif* 2022. However, the trait crude protein noticed significant positive and negative associations with the GD_{MOL} (0.244) and GD_{MOR} (-0.187) parents respectively. Geng *et al.*(2021) also reported negative association between GD_{MOL} of parents and their better parental heterosis for lint per cent and micronaire in cotton.

The GD_{MOR} assessed in parental genotypes showed significant negative relationship with cob height, plant height, crude protein and green fodder yield over both *kharif* 2022, *rabi* 2022 seasons. So, the prediction will increase by selection of parents with smaller GD than some threshold level (Melchinger (1999) and Betran *et al.* (2003) and it depends on the materials under the study (Melchinger *et al.*,1990).

Association between genetic distance of parents and standard parental heterosis: The correlation between standard heterosis performance with GD_{MOL} of parental inbreds was found to be significant for cob height (0.156)

and crude protein (0.152) in *kharif* 2022. But both of these traits do not register any significant associations in the *rabi* 2022. Refaee *et al.* (2016) also reported positive and negative relationship between GD_{MOL} of parents and standard heterosis of grain yield and productive panicle numbers respectively in rice.

The parental diversity (GD_{MOR}) displayed association towards standard heterosis for two traits. Days to fifty percent flowering was found to exhibit significant positive association with GD_{MOR} in both the seasons studied. However, consistent negative significant association was recorded for crude protein in kharif 2022, rabi 2022 (-0.128, -0.196). The low association between genetic distances of parents and their heterosis performance might be the effect of absence of linkage between genes controlling the traits studied, inadequate marker coverage over the genome, marker distribution and varied dominance effect (Melchinger et al., 1990; Charcosset et al., 1991; Kwon et al., 2002). Prediction could be enhanced further by the selection of more than 50 percent of markers linked with QTLs for the traits evaluated (Melchinger et al., 1990; Charcosset et al., 1991).

The greater $\mathrm{GD}_{_{\mathrm{MOL}}}$ and $\mathrm{GD}_{_{\mathrm{MOR}}}$ values of parental lines on mean green fodder yield and heterotic performance in superior performing crosses are furnished in Table 3. The correlation study revealed the non-significant impact of genetic distance on heterotic performance of hybrids. But when comparing the mean of green fodder yield of top performing hybrids with $\mathrm{GD}_{\rm MOL}$ and $\mathrm{GD}_{\rm MOR}$ of their parents, it is understood that the genetic distance had definite impact on identifying the hybrids with high green fodder yield and heterosis. During kharif 2022, the cross UMI 1200 X GETM 25 with parents of greater GD_{MOI} and GD_{MOR} exhibited higher green fodder yield with significant heterotic performance. Similarly, during rabi 2022 comparing top two crosses, N 66 X DM 12-4 had parents of higher GD_{MOR}, registered greater green fodder yield and superior standard heterosis.

Association analysis of fodder yield related traits in kharif 2022 and rabi 2022: The correlations between sixteen traits for kharif 2022, rabi 2022 seasons are presented in the Fig. 1. The trait days to fifty percent flowering was found to show significant positive association with most of the studied variables during kharif 2022 and rabi 2022. The lower diagonal is a result of Kharif 2022 and the upper one is the results of rabi 2022 and the size of the circle depicted denotes the strength of the correlation between traits. The trait crude protein had significant negative correlation with fifty percent flowering (-0.358) in kharif 2022. However, it does not show any positive relationship during the season Rabi 2022. It indicated that the prolonged flowering period is having a negative effect on crude protein content. Ahmet and Cengil, (2016) also reported that prolonged plant maturation causes decreased crude protein and digestibility in maize. The ADF (0.314) content in the Kharif 2022 and NDF (0.321) content in Rabi 2022 were found to possess positive relationship with days to fifty percent flowering and it was same as observed by Ahmet and Cengil, (2016) in maize. Plant height is an important component trait for increasing fodder yield and it had significant positive association with number of leaves, number of nodes, leaf length, internode length, stem girth, ADF and green fodder yield in both seasons. The current results were in corroboration with the reports of Manjeet Singh et al. (2022) on fodder sorghum.

The fodder yield contributing traits like plant height (0.299, 0.329), number of leaves (0.377, 0.354), number of nodes (0.377, 0.386), leaf length (0.405, 0.422) and dry matter yield (0.704, 0.638) were found to be positive and significantly correlated with fodder yield in both *Kharif* 2022 and *Rabi* 2022. During both the seasons crude protein content of plants was found to display significant negative correlation with other fodder quality traits like ADF (-0.643, -0.336) and NDF (-0.395, -0.222). Hence, these traits could be identified as constant traits, which maintain their persistent relationship with different

S.No.	kharif 2022													
	Cross with higher GFY	GD _{Mo∟} of parents	GD _{MOR} of parents	GFY (t/ha)	MPH (%)	BPH (%)	SH (%)							
1	UMI 1200 X GETM 25	0.63	7.58	80.21	103.71**	89.67**	20.88*							
2	UMI 1201 X TNFM 139-1	0.59	6.92	80.21	88.04**	59.43**	20.88*							
	rabi 2022													
1	N 66 X DM 12-4	0.58	6.16	71.92	127.72**	93.74**	67.79**							
2	N-09-160-2 X GETM 39	0.63	5.10	71.38	160.26**	141.66**	66.51**							

Table 3. Impact of greater GD_{MOL} and GD_{MOR} of parents on mean green fodder yield and heterosis of single cross hybrids

 GD_{MOL} - Genetic distance based molecular diversity, GD_{MOR} - Genetic distance based morphological diversity , GFY- Green fodder yield , MPH- Mid parent heterosis, BPH- Better parent heterosis, SH- Standard heterosis.



Bottom diagonal –*Kharif* 2022, Upper diagonal – *Rabi* 2022 Fig. 1. Association among 16 traits in single cross fodder maize hybrids over two seasons

component traits in different seasons. Ahmet and Cengil, (2016) reported that an increase in plant growth period increases the ADF and NDF content and reduces the protein content.

The ADF percentage of plants was recorded to be same in both the seasons with significant positive correlation with NDF content (0.668, 0.537respectively). The reason behind this might be NDF includes hemicellulose along with ADF fractions like cellulose, lignin and ash. Hence, both of these are highly correlated fractions and it corroborate well with the findings of Parmar *et al.* (2022) in forage bajra.

The crude fiber content of the hybrids had significant positive correlation with both ADF and NDF content in both the seasons studied. Crude fiber is the indigestible portion of feed that reduces digestion in ruminants. Crude fiber also possesses cell wall component like cellulose. Hence, this could be the main reason behind these positive associations with ADF and NDF. The detergent fibrous components like ADF had recorded significant correlation with green fodder yield (0.259) in *kharif* 2022 and it does not have any significant correlation during *rabi* 2022. Similarly, NDF was found to possess significant association in *rabi* season (0.147) but not in the *kharif* season. These results indicated that ADF and NDF have more interaction with the environment.

Although the correlation study revealed non- significant impact of genetic distance on heterotic performance of hybrids, when comparing the mean of green fodder yield of top performing hybrids with GD_{MOL} and GD_{MOR} of their parents, it is understood that the genetic distance had definite impact on identifying the hybrids with high green fodder yield and heterosis. It is evident from the cross UMI 1200 X GETM 25 with parents of greater GD_{MOL} and GD_{MOR} exhibited higher green fodder yield with significant heterotic performance. Similarly, N 66 X DM 12-4 had parents of higher GD_{MOR}, registered greater green fodder yield and superior standard heterosis. The present investigation revealed the existence of significant positive association between GD_{MOL} of parents and heterosis performance of crude protein and desirable significant negative correlation with crude fibre, ADF and NDF. Hence, selection of inbreds based on GD_{MOL} and GD_{MOR} would help the crop breeders to identify superior cross combinations with higher degree of heterosis for fodder traits.

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