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Exploring Genetic Variability for Health Benefiting Grain Nutritional Traits in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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ABSTRACT

In the present study variability parameters and association of grain mineral (Ca and Mg) with yield and its attributing traits were determined for fifty pearl millet genotypes at CCS, Haryana Agricultural University, Hisar during *kharif* 2020. Grain Ca content, grain yield/plant and dry fodder yield/plant were found to have high heritability along with genetic advance as percent of mean. A positive correlation was found for grain yield with 1000- seed weight but a negative and non-significant correlation with Ca and Mg content of grain indicating that these could be improved without compromising grain yield/plant. Highly significant and positive association between Ca and Mg content suggested that both these traits can be simultaneously improved. The lines viz., HMC-94-2, GP-69, GP-80, GP-70 and HMC-283 with high grain yield/plant (>30g) along with moderately high grain Ca (>115 mg/kg) and Mg (>1600 mg/kg) content were identified in present study.

Keywords Health-benefiting traits, Germplasm, grain Ca and Mg content, Pearl millet.

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INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.), the most heat-resilient and one of the highly drought- and salinity-resilient cultivated cereal is a most important food crop for resource poor farmers inhabiting in the semi-arid and arid tropics of Asia and sub- Sahara Africa (Haussmann *et al.* 2012). Earlier, termed as poor man's crop, it is now better known as nutri cereal benefitting crop. After maize (*Zea mays* (L.)), rice (*Oryza sativa* (L.)), wheat (*Triticum aestivum* (L.)), sorghum (*Sorghum bicolor* (L.) Moench) and barley (*Hordeum vulgare* (L.)), it is sixth most important cereal of the world. India is the leading producer of pearl millet in Asia. During the year 2020-21, the crop was cultivated in an area of 7.65 million ha with production and productivity of 10.8 million tons and 1420 kg/ha, respectively in India. In Haryana, area comprised by crop was 0.56 million ha with production and productivity of 1.3 million tons and 2372 kg/ha, respectively during the same year (Indiastat 2023).

Mineral deficiency has a serious impact on billions of people around the world, having an effect on their health and economic efficiency. Calcium and magnesium deficiency have serious health consequences. Insufficient intake of calcium lead to risk of osteoporosis and rickets, along with hypertension and stroke. Anorexia, muscle weakness and lethargy are symptoms of magnesium deficiency, which effects neuromuscular and neurologic function. There are several strategies to overcome malnutrition viz., industrial food fortification, dietary diversification and biofortification. Crop biofortification is of special significant for predominately agriculture based societies such as India since cultivars with elevated levels of minerals reach the rural households first and subsequently production surpluses penetrate the urban markets. Pearl millet is a very nutritious cereal with protein, minerals like Fe, Zn, P, Ca and Mg, essential amino acids and rich source of fiber. These characteristics make this crop greatly valued for everyday consumption (Satyavathi *et al.* 2015). Genetic variability is imperative to crop improvement. As society evolve, the need for food quality and quantity as well as other plant products are constantly changing. All these changes necessitate that plant breeder stay observant on regular basis for developing new cultivars. As a result, acknowledging existing variability in plant genetic resources is essential for the developing new cultivars with desirable traits. Pearl millet holds immense natural genetic and phenotypic variability for quantitative and qualitative traits (Bhattacharjee *et al.* 2007).

Grain minerals and yield are complex traits which are affected by the adjacent environment and are controlled by multiple genes (Owere *et al.* 2015). The correlation coefficient may help in determining whether characters are of major, minor or no significance in a breeding program. Therefore, a clear insight on genetic correlation of these minerals with grain yield and morphological traits can assist breeders in devising a suitable breeding stratergy for enhancement of minerals density in pearl millet grains. Path coefficient analysis, alternatively, provides information about direct and indirect effects of yield attributes in relation to yield. It depicts how the path values of attributing characters' influence yield, whether they affect yield directly or indirectly through influencing other interrelated characters. Heritability of a character confers an understanding into the heritable variance which can be transmitted to later generation. Evaluations of heritability, genetic advance as a percentage of mean, and their combination have also been considered as tools for envisaging the performance of the best selected lines in a population. In the recent past, major breeding efforts are focused on yield potential of crops and biofortification studies were primarily limited upto estimation of grain Fe and Zn contents. However, an estimation of other mineral such as grain Ca and Mg contents, their relationship with grain yield and yield contributing traits is also desirable for improvement of grain yield along with mineral content and not been performed. Therefore, present study was aimed to evaluate genetic variability and correlation and path coefficients among various morphological and nutritional traits in pearl millet.

MATERIALS AND METHODS

A total of 50 pearl millet genotypes (consisted of 9 B-lines and 41 R-lines) were evaluated in Randomized Block Design with two replications at research area of Bajra section, CCS Haryana Agricultural University, Hisar. Each genotype was grown in single row plot of 4-meter length with 45cm spacing between rows during *kharif* (Rainy) season of year 2020. All the recommended package of practices was adopted to raise a good crop of pearl millet. Observations were recorded for days to 50% flowering, panicle length (cm), panicle diameter (mm), number of productive tillers/plant, plant height (cm), dry fodder yield/plant (g), grain yield/plant (g) and 1000- seed weight (g). Representative soil samples were collected from 0-15 cm depth at pre-sowing and post- harvest stages of the crop. These samples were air dried, grounded and passed through 2.0 mm sieve to estimate available soil Ca and Mg by versenate titration method given by Cheng and Bray (1951) (Table 1). The open polli-

Table 1. Soil minerals status of experimental field, *kharif* 2020.

Location		Soil Ca (mg/kg)	Soil Mg (mg/kg)
Kharif 2020, Hisar	Pre sowing	14	14.4
	Post-harvest	6	9

Table 2a. Analysis of variance (ANOVA) for agro-morphological and biochemical characters in pearl millet germplasm lines.

		Mean sum of squares				
Source of variation (SV)	Degree of freedom (df)	Days to 50% flowering	Panicle length (cm)	Panicle diameter (mm)	Productive tillers (No./plant)	Plant height (cm)
Replication	1	1.96	1.61	5.66	0.01	18.14
Treatment	49	22.62**	23.72**	16.52**	0.41*	871.04**
Error	49	3.49	1.26	4.16	0.02	38.40

nated grains were thoroughly cleaned to remove any dust particles or other foreign materials. A known quantity (0.2g) of grain samples were digested using diacid mixture HNO_3: H_2O_2 in the ratio of 5:2 in closed microwave digestion system (MARS Xpress) at 170°C. After digestion final volume was made by adding ultra- deionized water. The diluted solution was filtered with the help of Whatman syringe filters (25mm diameter with 0.2µm pore size) and Ca and Mg concentration was estimated using ICP- MS (Inductively Coupled Plasma Mass Spectrometry, Thermo Fisher Scientific) and nutrient concentrations were given in mg kg^{-1}. ICP-MS is a sort of mass spectrometry which is able to detect several non-metals and metals at very low concentrations. Light and heat energy from high intensity plasma source ejects electron from shell (ionization) results into free electrons and atoms with positive charge (Ions). Ions are extracted and measured directly in mass spectrometer. Ultra- deionized water and ultrahigh-purity commercial acids (Sigma-Aldrich and Merck) were utilized to prepare all samples, reagents and standards. The calibration standards were made by diluting the stock multi-elemental standard solution (1000ppm) in 1% (v/v) nitric acid.

Statistical analysis

The replicated mean data was subjected to analysis for variance as per methods given by Panse and Sukhatme (1967). The estimates of genetic advance were calculated by the method given by Johnson *et al.* (1955). Genotypic (GCV) and phenotypic (PCV) coefficient of variations were estimated as per the method described by Burton and De vane (1953). Heritability in broad sense was calculated as the ratio of genotypic to the phenotypic variance and was illustrated in percent. Heritability was measured by the procedure suggested by Hanson *et al.* (1956). Simple correlation coefficients were calculated at genotypic and phenotypic levels among characters and were determined by following formula suggested by Al-Jibouri *et al.* (1958). Path analysis was performed using the simple correlation coefficients of yield and its components on yield to identify the direct and indirect effects of the yield and attributes as proposed by Wright (1921) and illustrated by Dewey and Lu (1959).

RESULTS AND DISCUSSION

For all morphological and biochemical characters examined in the present experiment, the analysis of variance (Tables 2a-2b) revealed highly significant differences among 50 different pearl millet germplasm lines, suggesting the existence of adequate variability. Large variability between germplasm lines was realised for the attributes like days to 50% flowering (52-67 days), panicle length (12.6-29.7cm), panicle

Table 2b. Analysis of variance (ANOVA) for agro-morphological and biochemical characters in pearl millet germplasm lines.

		Mean sum of squares				
Source of variation (SV)	Degree of freedom (df)	Grain yield/plant (g)	Dry fodder yield/plant (g)	1000- seed weight (g)	Grain Ca content (mg/kg)	Grain Mg content (mg/kg)
Replication	1	1.56	30.93	0.70	1,177.52	46,764.06
Treatment	49	136.13**	557.47**	3.71**	6,345.28**	2,59,972.65**
Error	49	3.76	17.53	0.61	98.77	2,515.80

* Significant at p= 0.05, ** Significant at p= 0.01.

Table 3. Estimates of genetic variability parameters (mean ± SE (m), range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, genetic advance and genetic advance as per cent of mean) for agro morphological and biochemical characters in pearl millet germplasm lines.

Sl. No.	Morphological traits	Mean ± SE(m)	Range	GCV (%)	PCV (%)	Heritability (h^2) (%)	Genetic advance as % of mean
1	Days to 50% flowering	62.16±1.3	52-67	4.97	5.81	73.26	8.77
2	Panicle length (cm)	20.98±0.7	12.6-29.7	15.90	16.83	89.92	31.17
3	Panicle diameter (mm)	27.00±1.4	20.05-33.65	9.20	11.91	59.71	14.65
4	Productive tillers (No./plant)	1.86±0.1	1.2-2.9	23.56	25.29	86.84	45.24
5	Plant height (cm)	154.25±4.3	117-187.5	13.22	13.82	91.55	26.07
6	Dry fodder yield/plant (g)	46.60±2.9	18.11-79.10	35.25	36.38	93.90	70.38
7	1000- seed weight (g)	9.38±0.5	6.72-11.67	13.26	15.68	71.51	23.10
8	Grain Ca content (mg/kg)	136.26±7.02	50.72-255.64	41.01	41.65	96.93	83.18
9	Grain Mg content (mg/kg)	1650.27±76.8	1063.65-2365.29	21.34	22.33	91.31	42.01
10	Grain yield/plant(g)	20.61±1.3	7.99-36.68	39.47	40.58	94.61	79.09

diameter (20.05-33.65 mm), number of productive tillers/plant (1.2-2.9), plant height (117-187.5 cm), grain yield/plant (7.99-36.68g), dry fodder yield/plant (18.11- 9.10g), 1000- seed weight (6.72-11.67g), grain Ca content (50.72-255.64 mg kg^{-1}) and grain mg content (1063.65-2365.29 mg kg^{-1}) in this study. Considerable variability for yield and its contributing was also stated by earlier workers in pearl millet germplasm accessions (Singh and Singh 2016, Sharma *et al.* 2018 and Govindaraj *et al.* 2020a).

For all traits, the phenotypic coefficient of variation (PCV) was found greater than the corresponding genotypic coefficient of variation (GCV), clearly depicting slight effect of environmental factors on trait expression and similar conclusion was made by the experiments of Kumar *et al.* (2014), Dhedhi *et al.* (2016) and Abdulhakeem *et al*. (2019). High values of GCV and PCV (Table 3) were obtained for traits like grain Ca content followed by grain yield/plant, dry fodder yield/plant, productive tillers/plant and grain Mg content suggesting variations for these traits provided significantly to the total variability and selection of these traits will be effective for their improvement. Similar results for PCV and GCV were also reported by Kaushik *et al*. (2018) in a set of 48 pearl millet maintainer lines for plant height, panicle length, number of productive tillers/plant and grain yield/plant. The higher GCV and PCV for grain Ca and Mg in germplasm accessions were recorded by Bashir *et al.* (2014) and Pucher *et al.* (2014). The traits viz., panicle length, plant height and 1000- seed weight had moderate GCV and PCV estimates in this study and in conformation with earlier reports by Kumar *et al.* (2014), Ramya *et al.* (2018) and Pallavi *et al*. (2020). However, days to 50% flowering displayed least GCV and PCV estimates and similar results were stated by Kumar *et al.* (2014), Kumari *et al*. (2018) and Ramya *et al.* (2018). Further, narrow range of difference between PCV and GCV in the experiment for all the morphological and biochemical traits indicated that if any selection pressure exerted on these traits, it might assist in improvement at early generations.

The estimates of heritability were high for traits viz., grain Ca content, grain yield/plant, dry fodder yield/plant, grain Mg content, plant height, panicle length, number of productive tillers/plant, days to 50% flowering and 1000-seed weight and moderate for panicle diameter (Table 3). Higher estimates of heritability for grain Ca and Mg content were earlier reported by Bashir *et al.* (2014) and Pucher *et al.* (2014) in germplasm accessions. High heritability for days to 50% flowering, panicle length and panicle diameter were earlier reported in pearl millet by Kumar *et al.* (2014).

The expected genetic advance as percent of mean was high for grain Ca content, grain yield/plant, dry fodder yield/ plant, number of productive tillers/plant, grain Mg content, panicle length, plant height and 1000- seed weight while moderate for panicle

Table 4. Estimates for genotypic and phenotypic correlation coefficients for morphological and biochemical traits in pearl millet germplasm lines.

Characters		Grain yield/plant (g)	Days to 50% flowering	Panicle length (cm)	Panicle diameter (mm)	Productive tillers (No./plant)	Plant heigh (cm)	Dry fodder yield/plant (g)	1000-seed weight (g)	Grain Ca content (mg/kg)
Days to 50% flowering	rg	-0.073								
	rp	-0.109								
Panicle length (cm)	rg	0.332**	0.292**							
	rp	0.319**	0.240**							
Panicle diameter (mm)	rg	0.258**	0.181	0.245*						
	rp	0.217*	0.096	0.282**						
Productive tillers (No./	rg	0.253**	-0.054	-0.134	-0.338**					
plant)	rp	0.259**	-0.118	-0.114	-0.159					
Plant height (cm)	rg	0.578**	0.130	0.071	0.054	0.273**				
	rp	0.575**	0.052	0.073	0.076	0.287**				
Dry fodder yield/plant (g)	rg	0.839**	0.163	0.314**	0.195	0.239**	0.536**			
	rp	0.835**	0.104	0.309**	0.185	0.244**	0.522**			
1000- seed weight (g)	rg	0.447**	0.275**	0.286**	0.458**	-0.051	0.384**	0.428**		
	rp	0.389**	0.215**	0.238**	0.396**	-0.038	0.332**	0.373**		
Grain Ca content (mg/kg)	rg	-0.073	-0.223*	-0.229*	0.123	0.071	-0.304**	-0.187	0.075	
	rp	-0.069	-0.177	-0.212*	0.099	0.045	-0.291**	-0.174	0.082	
Grain Mg content (mg/kg)	rg	-0.134	-0.131	-0.351**	-0.052	0.317**	-0.024	-0.187	-0.146	0.602**
	rp	-0.118	-0.093	-0.308**	0.027	0.310**	-0.020	-0.168	-0.103	0.562**

rg and rp are genotypic and phenotypic coefficient, respectively, * Significant at p = 0.05, ** Significant at p = 0.01.

diameter and was low for days to 50% flowering in this study. The results revealed that a high heritability trait does not always have to be complemented by a high genetic advance. However, in present study, high heritability together with high genetic advance as percent of mean was found for the traits viz., grain Ca content, grain yield/plant, dry fodder yield/plant, grain Mg content, plant height, panicle length, number of productive tillers/plant and 1000-seed weight clearly indicating the importance of additive gene action and the contribution of these traits to the total variability. Singh and Singh (2016) and Kaushik *et al*. (2018) also observed that number of productive tillers/plant, plant height, 1000- grain weight, grain yield/plant, had high heritability with high genetic advance which implies that selection will be more effective for these attributes. High heritability along with moderate and low genetic advance as percent of mean was recorded for panicle diameter and days to 50% flowering, respectively indicating occurrence of both additive and non-additive gene action. Similar results for days to 50% flowering was obtained by Kumari *et al*. (2018) and Pallavi *et al*. (2020) in pearl millet parental lines.

The results of correlation coefficient analysis (Table 4) showed that the attributes viz., panicle diameter, panicle length, plant height, number of productive tillers/plant, dry fodder yield / plant and 1000-seed weight exhibited significant positive association with grain yield/plant. Kumari *et al.* (2018) assessed fifty germplasm lines and recorded positive correlation of plant height and panicle length with grain yield/plant. Similar findings for 1000- grain weight, productive tillers/plant, panicle length, panicle diameter, plant height and fodder yield/plot in 13 parental lines were described by Bhasker *et al.* (2017) in his experiments. Sabiel *et al*. (2014) found that 1000- seed weight and plant height were exhibited positive correlation with grain yield/plant in pearl millet genotypes. Days to 50% flowering negatively and non-significantly correlated with grain yield/ plant and comparable results were also presented by Abuali *et al.* (2012). The finding was in also accord with reports given by Kumar *et al.* (2014), Ezeaku *et al.* (2015) and Talawar *et al.* (2017).

Results of present experiment showed that grain Ca and Mg content were negatively and non-sig-

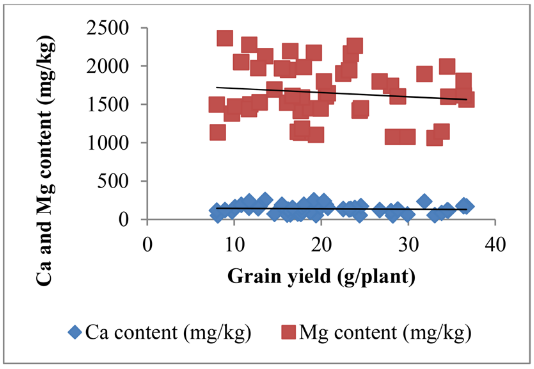

Fig. 1. Correlation between grain yield and grain minerals in pearl millet.

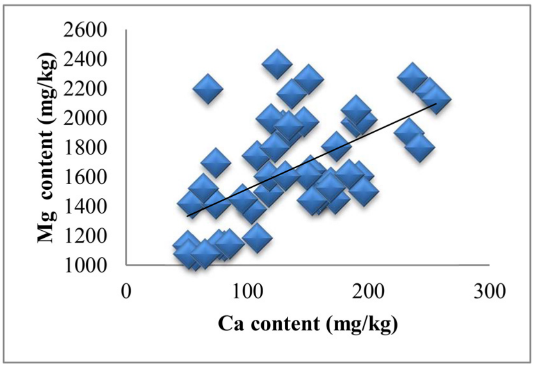

Fig. 2. Correlation between grain Ca and Mg content in pearl millet.

nificantly associated with grain yield/plant (Fig. 1.) Similar results for grain minerals and grain yield in pearl millet accessions were also presented by Bashir *et al.* (2014) and Pucher *et al.* (2014) in their studies. Lack of correlation of grain Ca and Mg content with grain yield and its component trait namely 1000-seed weight, panicle diameter and dry fodder yield in present study suggests that we can improve grain minerals content without affecting grain yield. Grain Ca was negatively and significantly associated with plant height and panicle length, while grain Mg content exhibited negative and significant correlation with panicle length. Bashir *et al.* (2014) reported that Ca and Mg negatively correlated with panicle length indicating that shorter panicles have high Ca and Mg content. The results in present investigation, illustrated significant and positive correlation of grain Ca and Mg content (Fig. 2) and similar results were reported by Pucher *et al*. (2014) and Govindaraj *et al.* (2020b). Therefore, positive correlation between grain Ca and Mg content indicates that both mineral traits can be simultaneously improved. In pearl millet, various reports indicate a highly positive correlation between grain Fe and Zn content as well Bashir *et al.* (2014) and Govindaraj *et al.* (2020b).

Using the path coefficient analysis approach, the estimated magnitude of genotypic correlation coefficients was partitioned to determine the direct and indirect effects, considering grain yield/plant as the effect (dependent variable) and other attributes as the causes (independent variables). In present experiment, dry fodder yield/plant had the greatest direct positive direct effect followed by plant height,

Table 5. Estimates of path coefficient analysis of grain yield/plant with its component characters in pearl millet germplasm lines.

Characters	Days to 50% flowering	Panicle length (cm)	Panicle diameter (mm)	Productive tillers (No./plant)	Plant height (cm)	Dry fodder yield/ plant (g)	1000-seed weight (g)	Grain Ca content (mg/kg)	Grain Mg content (mg/kg)	r_g (grain yield/ plant)
Days to 50% flowering	-0.267	0.047	0.023	-0.005	0.031	0.105	0.007	-0.026	0.010	-0.073
Panicle length (cm)	-0.078	0.163	0.031	-0.013	0.017	0.203	0.008	-0.027	0.027	0.332**
Panicle diameter (mm)	-0.048	0.040	0.129	-0.034	0.013	0.126	0.013	0.014	0.004	0.258**
Productive tillers (No./ plant)	0.014	-0.021	-0.043	0.101	0.066	0.154	-0.001	0.008	-0.024	0.253*
Plant height (cm)	-0.034	0.011	0.006	0.027	0.242	0.347	0.011	-0.036	0.001	0.578**
Dry fodder yield/plant (g)	-0.043	0.051	0.025	0.024	0.130	0.647	0.012	-0.022	0.014	0.839**
1000- seed weight (g)	-0.073	0.046	0.059	-0.005	0.093	0.276	0.028	0.009	0.011	0.447**
Grain Ca content (mg/kg)	0.059	-0.037	0.015	0.007	-0.073	-0.120	0.002	0.121	-0.047	-0.073
Grain Mg content (mg/kg)	0.034	-0.057	-0.006	0.032	-0.005	-0.121	-0.004	0.072	-0.078	-0.134

Residual effect- 0.169, r_g = genotypic correlation coefficient.

Table 6. Germplasm lines with high grain yield and moderately high grain minerals content.

Germplasm lines	Grain Ca content (mg/kg)	Grain Mg content (mg/kg)	Grain yield/ plant (g)	Days to 50% flowering	1000- seed weight (g)
HMC-94-2	168.87	1563.46	36.68	54	9.33
GP- 69	183.89	1617.85	36.36	60	11.67
GP- 80	173.45	1806.75	36.33	60	10.72
GP- 70	117.92	1603.20	34.58	60	10.27
HMC- 283	119.31	1994.35	34.45	63	9.64
HMC-94-4	85.29	1145.75	33.82	64	9.63
HR- 117	56.91	1063.65	33.01	63	9.96
HR- 1026	233.33	1898.60	31.81	52	10.55
HBL-21-10	64.90	1076.75	29.87	65	8.79
SGP- 10- 107-1	131.14	1605.49	28.77	58	7.67

panicle length, panicle diameter and grain Ca content on grain yield/plant (Table 5). Bhasker *et al.* (2017) showed that parameters like fodder yield/plot and panicle length exhibited the highest direct effect on grain production per plant. The findings were also endorsed by the results of Singh *et al.* (2015), Kaushik *et al*. (2018) and Kumar *et al.* (2020) for these characters. Consequently, it is advised that these traits can be used as major attributing traits in pearl millet breeding program to improve grain yield. Days to 50% flowering and grain Mg content exhibited direct negative effect on grain yield/plant. Estimates of indirect effects of various independent variables viz., dry fodder yield/ plant, plant height, panicle diameter and panicle length revealed high indirect effects on dependent trait (grain yield/plant). Low value of residual effects (0.169) depicted that the influence of various independent traits comprised in this investigation was high for grain yield/plant.

CONCLUSION

The present study revealed a comprehensive range of variability among pearl millet germplasm lines, indicating the potential for significant genetic gain through appropriate hybridization or selection. The positive association of morphological traits with the grain yield/plant clearly states that improving one or more of these attributes can lead to higher grain yield. Highly significant and positive association of grain Ca and Mg content and non-significant association of grain minerals with grain yield indicate that there are chances of getting lines with higher grain yield along with higher Ca and Mg content. Top ten high yielding germplasm lines were identified in the study having moderately high grain Ca and Mg content (Table 6). The study also identified contrasting germplasm lines having low and high values for grain Ca and Mg, which could be used as parental lines in future breeding programes for development of hybrids and to study their inheritance and in long run, mapping these traits through development of mapping populations. Direct selection for dry fodder yield/ plant and plant height shows substantial improvement in grain yield in germplasm lines of pearl millet. Furthest, the evaluated diversity can be used to widen the genetic base of genotypes in order to develop commercial hybrids.

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