MULTIVARIATE ANALYSIS FOR GRAIN NUTRITIONAL AND AGRO-MORPHOLOGICAL TRAITS IN PEARL MILLET [*PENNISETUM GLAUCUM* (L.) R. BR.]

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SUMMARY

The present study was carried out to assess genetic diversity for grain nutritional and agro-morphological traits among fifty genotypes (designated B and R-lines) of pearl millet. Significant genetic variation was identified for all the studied traits. Principal component analysis (PCA) was used to reduce large correlated data into three major principal components that report for maximum 65.04% of the variation. Among these PC1 contributes to the greatest variation (30%) followed by PC2 (19.95%.) and PC3 (15.08%). Maximum positive component loading towards PC1 was contributed by grain yield per plant (0.89), dry fodder yield per plant (0.85), number of productive tillers per plant (0.52) and towards PC2 was contributed by grain Ca content (0.84) and Mg (0.81) content. The biplot analysis revealed four groups with group IV having HR-108, HRI-1499, HR-1038, HR-1032 and GP-58 genotypes positively associated with grain Ca and Mg content and group I possessing GP-69, GP-80, GP-70, HMC-283 and HMC-94-2 genotypes positively associated to grain yield.

Key words: Biplot, minerals, pearl millet, principle component analysis, variability

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is one of the most important nutritious and food-security crops cultivated by marginal farmers in semiarid areas round the world. It is a climate-smart crop which can acclimatize to diverse range of environmental conditions, therefore is cultivated in high temperature, little and unpredictable rainfall and low soil fertility areas. It is a highly cross-pollinated crop due to protogynous mechanism, which satisfies one of the necessary biological prerequisite for development of hybrids. It is the most extensively used staple food crop, sustaining millions of people in Asia and sub-Saharan Africa (Fuller *et al.*, 2021). Pearl millet is the sixth most significant cereals crop globally after rice, wheat, maize, barley and sorghum (Patil *et al*., 2020). India is the world's largest producer of pearl millet in terms of area and production, where pearl millet is the fourth most extensively cultivated crop after rice, wheat and maize. In India, pearl millet occupied an area of 7.65 mha with production and productivity of 10.8 million tonnes and 1420 kg/ha, respectively in 2020-21 (Indiastat, 2023). Major pearl millet growing states which covers over 90% of pearl millet acreage in the country includes Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana. The area, production and productivity of pearl millet in Haryana in 2020-21 was 0.56 mha, 1.3 mt, and 2372 kg/ha respectively (Indiastat, 2023).

Micronutrient malnutrition can be expressed as inadequacy of one or more essential minerals and vitamins for human health. It results from inappropriate dietary composition, diseases and affect a large part of the population across world. Deficiencies of calcium and magnesium have detrimental effects on health. The contraction of muscles, transmission of nerve impulses, the growth of bones, cell signalling and blood clotting all requires calcium and magnesium (FAO, 2002). Calcium and magnesium deficiency is prevailing in both adults and children, primarily in developing countries (Combs and Nielsen, 2009). As pearl millet contains substantially larger quantities of grain minerals *viz.,* calcium, iron, zinc, and magnesium, lipids, high-quality proteins and energy value compared to all other major cereal food crops, it contributes significantly to the human diet and is considered as a potential crop to fight mineral inadequacies. It is an important source of fodder in many regions of the world. Forage obtained from pearl millet is a preferred feed for animals (Yadav *et al.,* 2023). Being gluten-free, it is widely used for patients with a gluten allergy. Pearl millet is an excellent source of energy (361 Kcal/100g), which provides more

energy than that of sorghum and comparable to that of brown rice, due to its generally higher lipid content, i.e. 3 to 6%. It consists of antioxidants that can be valuable to the generalised health and well-being (Nambiar *et al.,* 2011). The Government of India (GOI) has designated pearl millet and other millets as "Nutri-cereals" and declared 2018 as the Year of Millets in an attempt to boost and promote millet production.

Evaluation of genetic diversity and identifying superior genotypes are some of the basic objectives of any crop improvement programme. The widely diverse genotypes can be considered as parents to produce improved varieties/hybrids in a hybridization programme. Therefore, it is essential to take account of the genetic divergence among the germplasm. According to Moll and Stuber (1971), crosses among parents who are not closely related usually result in more heterosis than those between closely related ones. It is now feasible to choose parents with different genetic backgrounds due to the quantification of genetic variability through biometrical techniques. Principal component analysis (PCA) is the statistical techniques used to assess the amount of genetic diversity and evaluate the relative contribution of each studied attribute to the overall variation in grain yield (Phougat and Verma, 2022). Genetic variability and its consequent use are essential components in the production of novel cultivars by evaluating the main principal components and the key characteristics with highest positive or negative loadings in each component that determine the clustering pattern of diverse genotypes. Principal components analysis (PCA) is a data reduction method which can be applied to quantitative datasets that converts a set of multi-correlated variables into another set of uncorrelated ones (Bhatti *et al.* 2023).

A significant extent of variation for grain yield and associated attributes has been studied in pearl millet germplasm collections and commercially produced hybrids (Kanatti *et al.* 2014; Rai *et al.* 2013). However, there is relatively slight information available on the amount of variation for nutritional traits and diversity among commercially cultivated lines. A substantially greater extent of variation for these minerals has been identified in germplasm collections (Rai *et al.* 2014). A lot of breeding challenges from sustaining the higher yield potential with sufficient nutrition in breeding pipelines arise due to constant reduction in area and enhanced demand for nutritionally enriched grains. Therefore, breeding efforts must focus on

simultaneously enhancing of grain quality attributes along with grain yield. The production of nutrient-rich, high-yielding hybrids largely depends on the selection of diverse seed and pollen parents (Sharma *et al.,* 2020). In the light of this, present study was conducted to determine nature and extent of genetic diversity among germplasm lines for grain nutritional traits that serve as a base for selection of parents in pearl millet hybridization programmes.

MATERIALS AND METHODS

The present research was carried out during *kharif* (rainy) season 2020-21 at Bajra Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Geographically Hisar is sited at an altitude of 215.52 metres above mean sea level at 29°-10°N latitude and 75°-46°E longitude. Average weekly weather data of Hisar center, *Kharif,* 2020 is graphically presented in Fig. 1. The soil texture of investigational site is sandy loam in nature. It is located in semi-arid sub-tropical region which is present on the outer margins of the south-west (SW) monsoon region and has tropical monsoonal climate with mean annual rainfall of almost 452 mm. The material for the study comprised of 50 pearl millet genotypes (consist of designated maintainer and restorer lines of medium to late maturity) is listed in Table 1. The experiment was carried out in randomized block design with two replications. Each genotype was planted in single row plot of 4-meter length with row to row spacing of 45cm during *Kharif* (Rainy) 2020. Plant to plant distance was 10cm to 12cm. All the recommended set of practices was followed to produce good crop. A total of 8 quantitative traits *viz.,* days to 50% flowering, panicle diameter, panicle length, plant height, number of productive tillers per plant, dry fodder yield per plant, 1000-seed weight
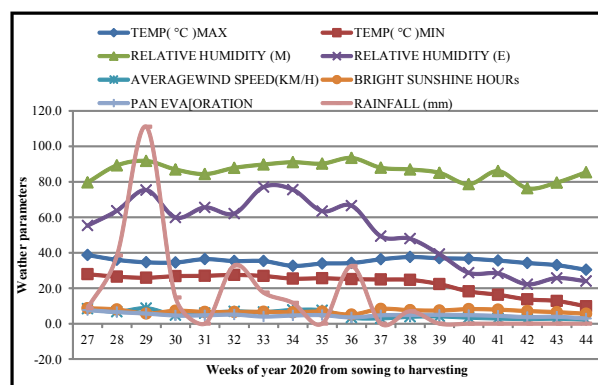

Fig. 1. Weekly weather data of Hisar, June-October, 2020.

TABLE 1
Description of pearl millet genotypes used in the study

S. No.	Genotype	Pedigree	S. No.	Genotype	Pedigree
1	HR-101	PDRL/16/101-1	26	HRI-13	EIHFEL/13
2	HR-607	ICRI 607	27	HRE-19	EERC-HS-29)-19-3
3	HR-117	TPRL/16/117	28	HMC-94-2	MC94C2-S1- 3-2-2-2-1-3-B-B × AIMP92901S1-488-2-1-1-1
4*	HBL-114	CPBL/16/114	29	HR-1038	PT-1-10-1038
5	HI-112	HFPL/16/112	30	HR-1026	TCF3-10-2-6
6	HI-1012	HFPL/1012	31	HR-1032	TCF3-10-3-2
7	HI-1013	HFPL/1013	32	HR-119	TPT-A2-1-11-119
8	HI-1037	HFPL/1037	33	HRI-115	High Fe JBT/12-115
9	HMC-94-4	MC94C2-S1-3-2-2-2-1-3-B-B × AIMP92901S1-488-2-1-1-4-B-B	34	GP-69	GP-69
			35	GP-70	GP-70
			36	GP-80	GP-80
10	HMC-94-28	MC94C2-S1-3-2-2-2-1-3-B-B × AIMP 92901S1-488-2-1-1-4-B-B)B-28-1-3	37	SGP-10-107-2	SGP-10-107-2
			38	EBL-12-237	EBL-12-237
			39	H78/711	H78/711
			40	EMRLT-14/103	EMRLT-14/103
11	ICMV91059	ICMV91059 S1-4-2-3-2-1-1-4-1-3-3-B-1	41	EMRL-14/243	EMRL-14/243
			42	ICMR09888	ICMR09888
12	HTRC	HTRC- Bulk-1084-1-5-4	43	HMC-283	MC94C2-S1-3-2-2-2-1-3-B-B ×
13*	HBL-21-10	ICMB02333 × ICMB92777)10-3-1-B			AIMP92901S1-488-2-1-1-4-B-B)-B-28-1-3
14*	HBL-21-5	ICMB92333 × EEBCC1-1)-5-B-B	44	ICRI1485	ICRI1485
15*	HB-1002-1	PT-1-10-1002	45	ICRI1499	ICRI1499
16*	HB-1124	PT-1-10-1124	46	SGP-10-107-1	SGP-10-107-1
17*	HB-1150	PT-1-10-1150	47	GP-61	GP-61
18	HTP03/13-927	HTP03/13-927	48	GP-62	GP-62
19*	HB-1002-2	PT-1-10-1002	49	GP-67	GP-67
20*	HB-116	LPRL-10-116	50	GP-58	GP-58
21*	HB-65	HB-65			
22	HRC-1171	HT Composite (B) 1171-1-3-1-4-3			
23	HR-108	EMRL/12-108			
24	HR-128	TCPTA/12-128			
25	HR-104	LPRL-10-104			

*B-lines and remaining lines are R-lines.

and grain yield per plant were taken on randomly chosen five competitive plants in each replication. When the main panicles of 50% of the plants in the row showed full stigma emergence, days to 50% flowering (days) was noted on the row basis.

Determination of mineral content

The open pollinated grains were thoroughly cleaned to remove any dust particles or other foreign materials. A known quantity (0.2 g) of grain sample was weighed, and put into a dry, cleaned and labelled digestion tube. 7ml of di-acid mixture HNO_3: H_2O_2 in the ratio of 5:2 was added into each digestion tube. The samples were then digested in closed microwave digestion system according to these conditions: a ramp time of 20 min to reach 170°C, a hold time of 25 min at 170°C and a cooling time of 15 min to reach 50°C. The samples were then allowed to cool at room temperature, they were vented and opened after which the solution was clear. After cooling, 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 concentrations were estimated using ICP- MS (Inductively Coupled Plasma Mass Spectrometry,

Thermo Fisher Scientific). Concentration of elements were determined by calibration curves and were given in mgkg⁻¹. All the reagents, standards, blanks and samples were prepared using ultra- deionized water and ultrahigh-purity commercial acids (Sigma-Aldrich and Merck).

Statistical analysis

To evaluate variability among genotypes, the quantitative data for each trait were examined for analysis of variance (ANOVA). To categorize influential components among studied quantitative traits principal component analysis (PCA) were evaluated. IBM SPSS Statistics version 26 was utilised to carried out the above-mentioned analyses on data collected for quantitative traits.

RESULTS AND DISCUSSION

In the present experiment, mean values for the grain nutritional and yield related traits (Table 2) varied significantly. Days to 50% flowering ranged from 52 to 67 days with mean value of 62.16±1.3days. Panicle length for different genotypes varied from 12.6 cm to 29.7cm with a mean of 20.98±0.7cm. Panicle diameter ranged from 20.0 mm to 33.6 mm with mean value of 27.00±1.4. An average value of productive tillers/plant was 1.8±0.1ranging from 1.2 to 2.9. Plant height varied from 117cm to 187.5cm with mean value of 154.25±4.3cm. Mean value of grain yield/plant of selected lines was 20.61±1.3g ranged from 7.99g to 36.68g. The trait dry fodder yield per plant exhibit significant variation ranged from 18.11g to 79.10g with a mean value of 46.60±2.9. Wide genetic variation from 6.72g to 11.67g with mean 9.38±0.5 was exhibited by the trait 1000- seed weight per plant. Mean value of grain Ca content of germplasm lines was 136.26 ±7.02 with a range of 50.72 mg/kg to 255.64 mg/kg. Grain Mg content varied from 1063.65mg/kg to 2365.29 mg/kg with average value of 1650.27±76.8

(Kavita et al., 2023). As the study material had a wide range of variability and acceptable mean performance for most of the traits explored and these lines can be selected and used as potential parents in various crop improvement programmes directed for simultaneous improvement of grain quality along with grain yield and other yield-attributing traits.

Principal Component Analysis is a multivariate analysis approach applied to reduce variability in various characters into smaller number of variables called as principal components, with the first principal component reflecting the greatest variability. The PCA derived from correlation matrix is best to evaluate interdependence among multiple traits and to define the principal factors because it does not comprise of the normal distribution assumption of populations (Chaudhary et al., 2015; Govindaraj et al., 2020; Kalagare et al., 2022; Sharma et al., 2020 and Tomar et al., 2021). The best principal components to describe system attributes included those that had greater eigen values (>1) and variables with high factor loadings. Total 10 principal components (PCs) were identified in the present study but only first three PCs are considered, as their eigen values were greater than one. The first three Principal components (PCs) considered for 65.04 per cent of the total variability for differentiating the genotypes of pearl millet based on grain nutritional, yield and its attributing components. The PC1 exhibited 30% of total variability followed by PC2 and PC3 which accounts for 19.95% and15.08% of total variability respectively (Table 3). The results showed that highest variability was distributed within first three principal components and PC1 exhibited the greatest variability among three. Therefore, it was suggested to consider the genotypes or traits showing more PC1 score and lying closer to PC1 for capturing the variability of desirable attribute.

TABLE 2

Mean values for agro - morphological and biochemical characters in pearl millet germplasm lines

S. No.	Morphological traits	Range	Mean ± S. E(m)
1.	Days to 50% flowering	52-67	62.16±1.3
2.	Panicle length (cm)	12.6-29.7	20.98±0.7
3.	Panicle diameter (mm)	20.05-33.65	27.00±1.4
4.	Productive tillers (No./plant)	1.2-2.9	1.86±0.1
5.	Plant height (cm)	117-187.5	154.25±4.3
6.	Dry fodder yield/plant (g)	18.11-79.10	46.60±2.9
7.	1000- seed weight (g)	6.72-11.67	9.38±0.5
8.	Grain Ca content (mg/kg)	50.72-255.64	136.26±7.02
9.	Grain Mg content (mg/kg)	1063.65-2365.29	1650.27±76.8
10.	Grain yield/Plant(g)	7.99-36.68	20.61±1.3

TABLE 3

Eigen values of different quantitative traits in pearl millet

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	3.000	30.003	30.003
2	1.995	19.954	49.956
3	1.509	15.089	65.046
4	0.964	9.637	74.683
5	0.756	7.560	82.244
6	0.543	5.425	87.669
7	0.493	4.930	92.599
8	0.402	4.025	96.624
9	0.230	2.303	98.927
10	0.107	1.073	100.000

Kumar *et al.* 2020 evaluated that first four principle components contributed to 68.3 % variance in pearl millet genotypes. Similarly, Ton *et al.* (2022) stated that the first three principal components described 76.3% of variability for agro-morphological traits and 69.6% of collective variance for mineral concentration in local pea genotypes.

Based on the factor loading of PCA analysis, it was found that PC1 contributed highest component loading for grain yield per plant (0.89), dry fodder yield per plant (0.85), plant height (0.76) and number of productive tillers per plant (0.52). In the second principal component highest positive component loading accounted by grain Ca content (0.84) and grain Mg (0.81) content and the maximum negative component loading contributed by days to 50% flowering (0.46) and panicle length (0.44). The panicle diameter (0.82) and 1000-seed weight (0.65) with highest positive component loading and number of productive tillers per plant (0.46) with maximum negative component loading were contributed in the third principle component (PC3) (Table 4). Result of factor loading of PCA evaluation showed that the greatest variability contributed by the PC1 was significantly associated to most of the yield attributing traits and PC2 exhibited maximum factor loading for grain nutritional traits. Ton *et al.* 2022 also reported positive association of grain Ca and Mn content with PC2 in their studies. They also described positive association of PC1 with K, Zn, Mg, P, Cu and protein. Traits which exhibited high positive or high negative loadings added more to the diversity. The sign here represents the relation between principal components and variable (Rasitha *et al.,* 2020; Kalagare *et al.,* 2022 and Mawouma *et al.,* 2022). PC1 and PC2 showed

negative factor loading for days to 50% flowering (-0.24) indicating the negative correlation with the trait.

PCA results are usually shown as a biplot, where axes relate to the new system of coordinates (Fig. 2). The direction of arrow signifies the higher variation and the length could be associated with the rate of change occur. The acute coordinate angle (<90 °) among the attributes or between principal component axis and attribute displays the positive correlation among these attributes, whereas obtuse angle (>90º) indicate negative and right angle (=90º) represents no correlation between the traits (Govindaraj *et al.,* 2020; Pujar *et al.,* 2020 and Sharma *et al.,* 2022). On the basis of trait biplot, four groups of characters were identified, among which the first group was composed of number of productive tillers and grain yield per plant which exhibited positive correlation with the first two PCs. The second group was negatively correlated with PC2 and comprised of plant height, dry fodder yield per plant, 1000-seed weight, panicle diameter and panicle length. Third group contained days to 50% flowering that was negatively associated with both the components (PC1 and PC2). The fourth group contained grain Ca content and grain Mg content that was negatively correlated with PC1. Most of the attributes be present in acute angle with PC1 axis except grain Ca content, grain Mg content and days to 50% flowering. Both grain Ca and Mg content were in acute angle with PC2 which depicted their highest contribution towards this component. The lowest acute and adjacent angle with grain yield per plant were found for plant height and dry fodder yield per plant followed by 1000-seed weight, panicle diameter and panicle length showing positive association and displayed the significance of the variable selection for improvement of yield attributing traits. However, grain Ca content, grain Mg content and days to 50% flowering was negatively correlated with grain yield per plant, as depicted by the obtuse angle between them. Grain Ca were in acute angle with grain Mg content which show their positive association with each other. The genotypes *i.e.* GP-69, GP-80, GP-70, HMC-283 and HMC-94-2 having positive values for both main components (first group) in the biplot (Fig. 2) would contribute to more towards the yield. Similarly, HR-108, HRI-1499, HR-1038, HR-1032 and GP-58 genotypes having negative value for the PC1 (fourth group) in biplot may be utilised as potential parents in breeding programmes for enhanced grain minerals content. The present experiment was in conformity with the PCA traits analysis of Gerrano *et*

TABLE 4

Factor loading of different characters with respect to principal factor in pearl millet

| Rotated Component Matrix	Component		
	1	2	3
Grain yield/plant (g)	0.893	-0.003	0.224
Dry fodder yield/Plant (g)	0.851	-0.138	0.208
Plant height (cm)	0.762	-0.126	-0.005
Productive tillers (No./plant)	0.522	0.366	-0.464
Grain Ca content (mg/kg)	-0.191	0.848	0.246
Grain Mg content (mg/kg)	-0.073	0.816	-0.056
Days to 50% flowering	-0.242	-0.464	0.332
Panicle diameter (mm)	0.066	0.063	0.829
1000- seed weight (g)	0.428	0.033	0.653
Panicle length (cm)	0.219	-0.449	0.471
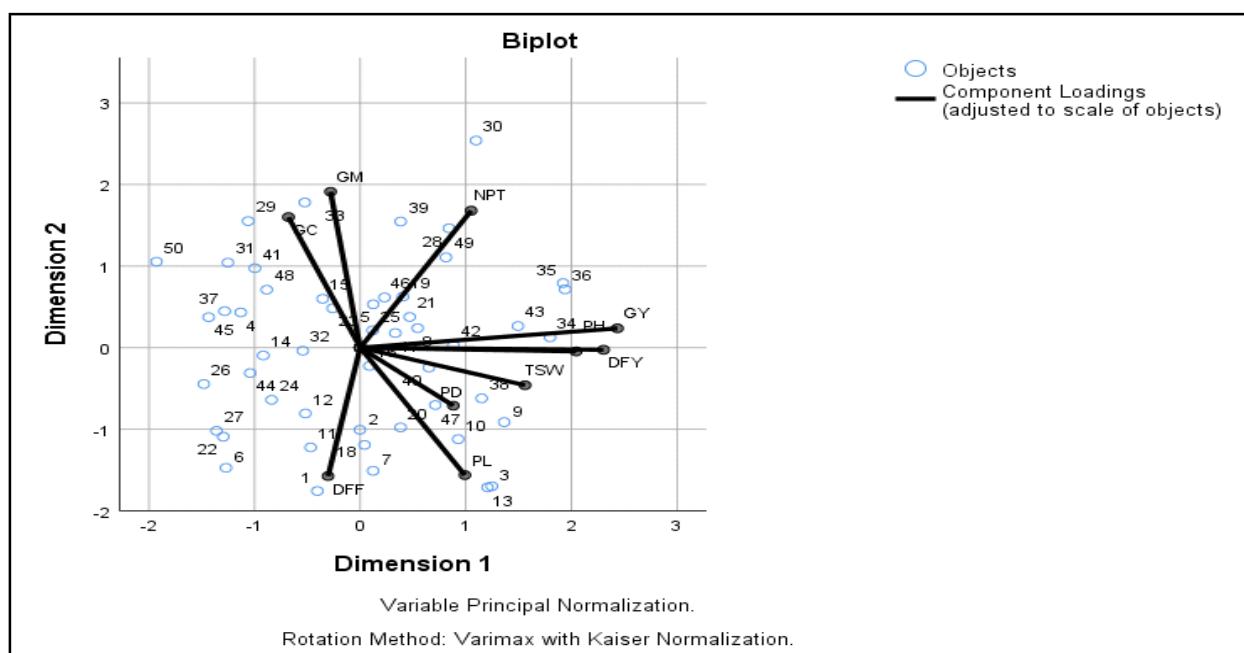
Fig. 2. Biplot showing relation between grain mineral, yield and its attributing traits of pearl millet lines on first two principle components.

DFF- Days to 50% flowering, PL- Panicle length, PD- Panicle diameter, PH- Plant height, NPT- Number of productive tillers per plant, DFY- Dry fodder yield per plant, GY- Grain yield per plant, TSW- 1000-seed weight, GC- Grain Ca content, GM- Grain Mg content.

al., 2018; Kumar *et al.,* 2020; Govindaraj *et al.,* 2020; Tomar *et al.,* 2021; and Kalagare *et al.,* 2022; Triki *et al.,* 2022 and Mawouma *et al.,* 2022.

CONCLUSION

Based on mean performances, high grain Ca (>180mg/kg) and Mg (>2000mg/kg) content was recorded in germplasm lines *viz.,* HR-108, HR-1032, HRI-115, ICRI-1499, GP-58 and GP-227. Inbreds identified with high level of mineral content (Grain Ca and Mg) may utilized directly or indirectly in breeding pearl millet biofortification programme. Principal component analysis (PCA) assists in classifying genotypes diverse in terms of variability which is extremely valuable in crop improvement The genotypes HMC-94-2, GP-69 and GP-80 having positive values for both main components would contribute to more towards the yield and genotypes HR-108, HRI-1499, HR-1038 having negative value for the both major components are rich in grain nutritional traits hence, these genotypes can be used in future such as parent genotypes for desirable traits.

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