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# Genetic diversity amongst oat (*Avena sativa*) lines for micronutrients and agro-morphological traits

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ABSTRACT

The present experiment was conducted during winter (*rabi*) seasons of 2019–20 and 2020–21 at the ICAR-Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh to study the genetic diversity amongst oat (*Avena sativa* L.) lines for micronutrients and agro-morphological traits. For study, 150 oat accessions collected from different sources were evaluated for two years and four micronutrients (Zn, Cu, Fe and Mn) and 9 agro-morphological traits were recorded. Genotypes IG02122 (464.0 mg/kg), IG02156 (48.1 mg/kg), IG03271 (136.0 mg/kg), and IG03213 (22.0 mg/kg) had maximum Fe, Zn, Mn and Cu content in fodder (harvested at 50% flowering). Genotype IG0280 had both high Zn (36.97 mg/kg) and Mn (114.33 mg/kg); IG03233 had high Cu (18.0 mg/kg) and Mn (124.0 mg/kg); and IG02131 had high Cu (18.33 mg/kg) and Fe (369.0 mg/kg) content. Analysis of variance (ANOVA) highlighted significant genotypic differences ($P$<0.001) for micronutrient content and fodder yield and related traits. High heritability coupled with high genetic advance was found for micronutrients, green fodder yield, test weight, dry matter yield, plant height, tiller number and grain number suggested the preponderance of additive and fixable genetic variance for these traits. The Cu content had significant negative association with Mn content but positive with leaf length and leaf width. Principal component analysis separated the total genetic variation into five main components and covered 59.09% of the total genetic variation. Based on Mahalanobis distances, genotypes were grouped into six clusters where maximum inter-cluster distance was observed for cluster 4 and 5. Therefore, genotypes from these two clusters can be used as parents for the future breeding programmes.

**Keywords**: Correlation, Fodder oat, Genetic diversity, Genetic parameters, Micronutrients

India's livestock sector is one of the largest in the world with the total livestock population of 535.78 million and accounts nearly 20% of world's total cattle population (Anonymous 2019). Livestock sector contributes 4.11% in GDP and 25.6% of the total agriculture GDP (Anonymous 2019). Food and cash crops cover the maximum area under arable land, whereas fodder crops are cultivated in less productive, water scarce and marginal lands (Chand *et al.* 2022). To meet the current fodder demand, India must produce approximately 53.19 million tonnes (Mt) however, only 22.74 Mt is being produced that reflects the vast gap between fodder demand and supply (Dhaliwal *et al.* 2020). Besides, nutritional security is an equally important for livestock as for human beings and fodder crops with high mineral content value could be one of the way to meet the livestock mineral demand.

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Forage-derived minerals are essential for animal health, reproduction, vigor and increase the end-product nutritive value (Chand *et al.* 2022, Sood *et al.* 2022). Oat (*Avena sativa* L.; 2n=6x=42) is used for multi-purposes, viz. food, feed and nutraceutical for its unique nutritional value (Boczkowska *et al.* 2016, Rosentrater *et al.* 2018). Oat as a livestock feed has a unique nutritional value and consists relatively high quality protein. Indian soils are deficient of zinc (Zn), copper (Cu), and manganese (Mn) by 49, 4 and 3%, respectively of soil samples (Devi *et al.* 2014). Oat is the major source of dry fodder but low in Zn concentrations, particularly when grown on Zn deficient soil of India. Besides, Cu and Zn play vital role in metabolic activities in animal system and their continuous supply in animal's diet is essential to maintain healthy growth, immunity and productivity (Haenlein 2004). Deficiency of Zn reduces feed intake, growth, listlessness, excessive salivation, testicular growth, cracked hooves, fertility and skin lesions or slowed wound healing in animals (White and Brown 2010). Food crops, used as livestock feed especially rice, wheat, maize and oat, are the major source of roughages for animals, but they are inherently low in nutrient concentrations, particularly

when grown on nutrient-deficient soil (Godara *et al.* 2019). Development of high yielding biofortified fodder crops can meet both feed and nutritional security of livestock sector in the country.

With this background, present study was designed to evaluate 150 oat genotypes for four micronutrients, viz. Zn, Fe, Cu and Mn content in fodder along with fodder yield and yield contributing traits; correlation between micronutrients and growth parameters; contribution of minerals and growth parameters to genetic diversity; and clustering of genotypes for their use in future breeding program, particularly developing bio-fortified varieties.

## MATERIALS AND METHODS

The present experiment was conducted during winter (*rabi*) seasons of 2019–20 and 2020–21 at ICAR-Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh. A set of 150 oat genotypes, a collection of the USA, Sweden and India consisting of released varieties, registered germplasm and exotic genotypes, were used for the study (Supplementary Table 1). The experiment was laid out in randomized complete block design (RCBD) with three replications. The row to row and plant to plant distance was 50 cm × 5 cm, respectively apart. For micronutrient analysis, plant samples were subjected to metal analysis as per the method of Association of Official Analytical Chemists (AOAC 2005). Plant samples were collected at 50% flowering stage of the plant and oven-dried for two days at 60°C. After drying, the material was crushed in a Wiley type mill with a sieve of 2 mm. The grounded samples were then processed through wet-digestion using tri-acid mixture (nitric acid, perchloric acid and sulphuric acid, in the ratio of 1.5:2:3). For the determination of micronutrients (Zn, Cu, Fe and Mn), 1 g dried plant sample was taken in conical flask, added 10 ml tri-acid mixture and digested on a hot plate till the material was clear. After digestion, the material was cooled and the digest was filtered with Whatman filter paper number 42, the content was transferred into 50 ml volumetric flask and the volume was made up with double distilled water (Miller *et al.* 1998). 1 ml solution from 50 ml was further diluted to 20 ml using double distilled water that was ready for estimation of Zn, Cu, Fe and Mn through Atomic Absorption Spectrophotometer (AAS) and results were expressed in mg/kg sample. A set of standard solutions of Fe, Zn, Cu, and Mn within the analysis range was run before and after each set of 10 test solutions. Samples were analyzed in duplicate, and the analysis was repeated if results differed by more than 10%. The variation in replications for each sample did not exceed ± 2 ppm for Zn and Cu, ± 5 ppm for Fe and ± 4 ppm for Mn.

In the present investigation, 9 morphological traits were recorded at appropriate crop growth stage on the field. 10 representative plants from each replication were selected and tagged for recoding the data at suitable stages. The morphological parameters, viz. plant height (PH) (cm); number of leaves (NL); leaf length (LL) (cm); Leaf width (LW) (cm); number of tiller (TN); number of grains/plant (GN); 100-seed weight (TW) (g); green fodder yield (GFY) (kg/m$^2$) and dry matter yield (DMY) (kg/m$^2$) were recorded. The genotypic (GCV) and phenotypic (PCV) coefficient of variation were estimated as per the procedure of Burton and Devane (1953). Likewise, broad sense heritability ($h^2b$), genetic advance (GA) and genetic gain over the percent of mean ($GG_m$) were calculated using the procedure of Johnson *et al.* (1955). The correlation between and within estimated and recorded traits, principal component analysis (PCA) and clustering of genotypes into different groups were analyzed by R software (R Core Team, 2022).

## RESULTS AND DISCUSSION

*Per se performance of genotypes for micronutrients and other traits*: Micronutrient profiling indicated that both Fe (mean: 229.54 mg/kg) and Mn (mean: 71.13 mg/kg) content were more prevalent in all studied accessions than Zn (mean: 27.43 mg/kg) and Cu (mean: 9.88 mg/kg) (Table 1). The Fe, Zn, Mn and Cu content were ranged from 30.0 (IG02115) to 464.0 (IG02122), 13.0 (IG02114) to 48.1 (IG02156), 17.0 (IG02125) to 136.0 (IG03271) and 1.0 (IG0266) to 22.0 (IG03213) mg/kg, respectively. Besides, 4 accessions had >40.0 mg/kg Zn content, 16 had >16.0 mg/kg Cu content, 9 had >365.0 mg/kg Fe content and 16 genotypes had >106.0 mg/kg Mn content in fodder (Table 1). Two genotypes of each lowest and highest micronutrient content of Fe, Zn, Cu and Mn (Fig. 1) and contrasting genotypes could be used in breeding programmes for developing breeding populations for tagging and molucular mapping of the trait of interest. Besides, genotype IG0280 had both high Zn (36.97 mg/kg) and Mn (114.33 mg/kg); genotype IG03233 had high Cu (18.0 mg/kg) and Mn (124.0 mg/kg); and genotype IG02131 had high Cu (18.33 mg/kg) and Fe (369.0 mg/kg) content. The accessions collected from the USA had the highest Zn, Fe and Cu content, whereas highest Mn content line was from Sweden. Besides, wide range of phenotypic variability for micronutrients was observed among the oat accessions collected from different locations. Likewise, morphological traits also showed wide phenotypic variation ranging from 75 (IG03458) to 198 cm (IG03469) for PH, 3 (IG03255) to 10 (IG03272) for NL, 5 (IG03273) to 54 cm (IG02115) for LL, 1 (IG02142) to 7 cm (IG0299) for LW, 1.9 (IG0272) to 26 (IG03275) for TN, 12 (IG02146) to 195 (IG02172) for GN, 1.1 (IG03276) to 4.8g (IG02148) for TW, 0.21 (IG0297) to 4.8 kg/m$^2$ (IG03460) for GFY and 0.08 (IG02101) to 0.98 kg/m$^2$ (IG03460) for DMY.

*Analysis of variance and genetic parameters*: Analysis of variance (ANOVA) indicated significant genotypic differences ($P$<0.001) for micronutrient content and fodder yield and related traits among 150 oat accessions (Table 2). A significant genetic variation for the studied traits amongst the genotypes could be used for the selection of superior genotypes through direct phenotypic selection (Kumar *et al.* 2021, Sharma *et al.* 2022). For micronutrients, the magnitude of genetic variance ($V_g$) was greater over the environmental variance ($V_e$) indicating that genetic constitution had major control for micronutrient expression over the environmental

Table 1 Grouping of 150 oat accessions based on their fodder micronutrient profiling

| Micronutrient | Concentration (mg/kg) | Number of genotypes | Genotypes |
|---|---|---|---|
| Zn content | <20.0 | 9 | 78, 36, 38, 24, 27, 40, 106, 13, 39 |
| | 20.0–40.0 | 137 | 31, 126, 1, 60, 92, 21, 141, 142, 88, 35, 9, 19, 69, 87, 130, 93, 99, 96, 23, 6, 14, 34, 3, 59, 81, 68, 4, 32, 48, 42, 56, 136, 49, 71, 26, 122, 29, 144, 75, 98, 64, 33, 58, 63, 73, 67, 115, 145, 138, 43, 52, 66, 8, 80, 45, 70, 37, 82, 20, 72, 137, 51, 114, 135, 140, 18, 57, 129, 146, 65, 127, 124, 97, 86, 102, 62, 76, 111, 104, 16, 61, 30, 74, 95, 109, 113, 91, 107, 54, 84, 105, 53, 121, 90, 132, 149, 77, 103, 143, 50, 110, 83, 94, 148, 46, 22, 7, 25, 101, 119, 112, 85, 55, 41, 128, 147, 139, 12, 108, 117, 44, 150, 5, `133, 118, 123, 47, 134, 79, 100, 120, 125, 28, 131, 15, 116, 89 |
| | >40.0 | 4 | 10, 17, 11, 2 |
| Cu content | <7.0 | 57 | 62, 5, 64, 9, 12, 25, 43, 10, 19, 70, 99, 40, 41, 7, 4, 8, 105, 27, 45, 46, 77, 82, 15, 23, 72, 11, 2, 3, 16, 21, 22, 24, 35, 100, 14, 17, 26, 29, 32, 36, 39, 47, 128, 135, 136, 13, 34, 38, 42, 65, 71, 80, 88, 28, 57, 106, 145 |
| | 7.0–16.0 | 77 | 6, 31, 44, 50, 101, 33, 102, 124, 30, 97, 52, 75, 89, 120, 123, 1, 58, 122, 114, 48, 83, 131, 49, 86, 104, 116, 95, 127, 144, 147, 92, 132, 93, 125, 69, 91, 98, 117, 134, 143, 74, 81, 121, 55, 90, 130, 142, 73, 60, 67, 84, 109, 110, 96, 111, 126, 138, 139, 59, 68, 85, `133, 137, 148, 61, 54, 78, 79, 107, 140, 76, 108, 112, 87, 118, 146, 149 |
| | >16.0 | 16 | 18, 94, 20, 53, 129, 141, 56, 63, 113, 150, 51, 37, 66, 103, 119, 115 |
| Fe content | <169.0 | 38 | 79, 85, 21, 103, 87, 75, 51, 63, 57, 89, 74, 100, 54, 122, 136, 45, 30, 126, 70, 4, 82, 124, 6, 5, 129, 17, 76, 101, 1, 52, 43, 95, 102, 106, 116, 86, 28, 84 |
| | 169.0–365.0 | 103 | 71, 142, 34, 119, 77, 14, 44, 47, 20, 33, 130, 78, 117, 145, 128, 110, 104, `133, 65, 59, 50, 121, 97, 41, 2, 144, 55, 118, 131, 96, 109, 48, 94, 99, 107, 39, 46, 92, 143, 31, 125, 72, 139, 127, 27, 141, 37, 115, 26, 60, 68, 36, 49, 11, 19, 10, 69, 80, 13, 90, 123, 146, 120, 105, 81, 83, 23, 62, 108, 64, 53, 35, 113, 112, 150, 56, 8, 93, 16, 135, 22, 25, 147, 15, 32, 132, 18, 138, 98, 29, 111, 7, 40, 42, 3, 148, 9, 137, 73, 91, 140, 114, 88 |
| | >365.0 | 9 | 66, 149, 38, 134, 12, 58, 61, 24, 67 |
| Mn content | <47.0 | 31 | 70, 92, 66, 125, 75, 96, 60, 142, 116, 111, 143, 58, 113, 59, 102, 119, 12, 56, 91, 101, 128, 146, 139, 63, 137, 95, 110, 68, 79, 130, 144 |
| | 47.0–106.0 | 106 | 88, 57, 81, 78, 82, 126, 100, 109, 123, `133, 77, 17, 36, 55, 103, 129, 115, 108, 134, 97, 76, 112, 8, 89, 121, 67, 4, 6, 65, 106, 72, 117, 20, 136, 39, 107, 132, 114, 124, 24, 61, 19, 38, 16, 3, 7, 13, 122, 71, 33, 141, 25, 11, 64, 93, 120, 127, 149, 29, 94, 135, 22, 87, 37, 147, 40, 32, 9, 73, 35, 21, 150, 10, 44, 145, 31, 74, 27, 49, 54, 104, 1, 80, 34, 118, 148, 86, 2, 23, 5, 18, 69, 42, 45, 47, 105, 131, 52, 41, 53, 84, 43, 90 |
| | >106.0 | 16 | 98, 14, 15, 62, 99, 30, 28, 140, 83, 85, 51, 138, 46, 48, 26, 50 |

variations (Table 2). For morphological traits, the magnitude of $V_g$ was higher than $V_e$ except NL but in different proportion, therefore, environmental factors had also played a role in trait expression. The Fe, Mn content, GN and PH were recorded high genetic variance whereas DMY, LW and TW had the lowest value. Traits such as GFY, DMY, TW, Zn content, and LW had the minimal gap between phenotypic and genotypic variance suggesting that traits are more stable and least influenced by the environmental conditions.

The GCV and PCV have role in the expression of traits and determine the magnitude of genetic and environmental effects. The GCV and PCV values were almost equal for micronutrients, TW and GFY indicating that environmental variation had limited effect on these traits (Table 2). The GFY, DMY, GN, Cu, Mn, Fe content, and TN had high GCV and PCV values whereas low values were reported for PH, NL, Zn content, TW, LL and LW. The higher value of GCV and PCV indicated that large variation was present among the genotypes for GFY, DMY, GN, Cu, Mn, Fe content and TN, therefore, direct selection could be applied for breeding programmes. Likewise, high GCV and PCV values for GFY, DMY, NL, and TN was reported by Toppo and Sahu (2022) in oat genotypes. In the present study, a high level of broad sense heritability (>0.60) was recorded for micronutrients, GFY, TW, DMY, PH, TN and GN; moderate (0.40–0.60) for NL, LL, and low (<0.40) for LW. High genetic advance (>20%) as per cent of mean was expressed for the studied micronutrients and fodder yield related traits except NL that showed moderate level (10–20%) (Table 2). However, Toppo and Sahu (2022) reported high genetic advance for 1000 seed weight, moderate for GFY/plant and low for PH, NL/plant, TN/plant, DMY/plant. High value of heritability accompanied by high estimate of genetic advance as percent of mean expressed by micronutrients, GFY, DMY, TW, TN, PH and GN suggested the preponderance of additive and fixable genetic variance for these traits. Therefore, phenotypic selection methods could be exploited for

Table 2 Analysis of variance (ANOVA), descriptive statistics and genetic variability parameters for forage micronutrients, fodder yield and related traits among 150 oat genotypes

| Character[+] | Mean sum of square[@] | | | Descriptive and genetic parameters[$] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rep. (2) | Genotypes (149) | Error (248) | Range | Mean | SEm | CD ($P$=0.05) | CV | $V_e$ | $V_g$ | $V_p$ | ECV | GCV | PCV | $H^2b$ | GA | GA(m) |
| Zn | 0.02 | 91.25*** | 0.16 | 13.0-48.1 | 27.43 | 0.23 | 0.64 | 1.45 | 0.16 | 30.37 | 30.52 | 1.44 | 20.09 | 20.14 | 0.99 | 11.32 | 41.27 |
| Cu | 3.39** | 67.96*** | 0.55 | 1.0-22.0 | 9.88 | 0.43 | 1.19 | 7.50 | 0.55 | 22.47 | 23.02 | 7.50 | 47.97 | 48.55 | 0.98 | 9.65 | 97.63 |
| Fe | 109.8*** | 19280*** | 9.80 | 30.0-464.0 | 229.54 | 1.81 | 5.04 | 1.37 | 9.83 | 6423.38 | 6433.22 | 1.37 | 34.92 | 34.94 | 1.00 | 164.97 | 71.87 |
| Mn | 10.75*** | 2151.75*** | 1.30 | 17.0-136.0 | 71.13 | 0.66 | 1.83 | 1.60 | 1.30 | 716.82 | 718.11 | 1.60 | 37.64 | 37.67 | 1.00 | 55.10 | 77.47 |
| PH | 502.07** | 870.63*** | 95.36 | 75.0-198.0 | 132.52 | 5.64 | 15.69 | 7.37 | 95.36 | 258.42 | 353.79 | 7.37 | 12.13 | 14.19 | 0.73 | 28.30 | 21.36 |
| NL | 0.44 | 1.94*** | 0.56 | 3.0-10. | 5.44 | 0.43 | 1.21 | 13.78 | 0.56 | 0.46 | 1.02 | 13.78 | 12.42 | 18.56 | 0.45 | 0.93 | 17.14 |
| LL | 113.72** | 92.82*** | 21.88 | 5.0-54.0 | 23.41 | 2.70 | 7.52 | 21.64 | 21.88 | 23.65 | 45.53 | 19.98 | 20.77 | 28.83 | 0.52 | 7.22 | 30.84 |
| LW | 0.21 | 0.53*** | 0.18 | 1.0-7.0 | 2.10 | 0.24 | 0.68 | 20.05 | 0.18 | 0.12 | 0.29 | 20.05 | 16.18 | 25.76 | 0.39 | 0.44 | 20.92 |
| TN | 14.43 | 45.79*** | 6.88 | 1.9-26.0 | 10.01 | 1.51 | 4.21 | 26.21 | 6.88 | 12.97 | 19.85 | 26.21 | 35.98 | 44.51 | 0.65 | 6.00 | 59.92 |
| GN | 1149.61** | 1833.96*** | 216.87 | 12.0-195.0 | 54.57 | 8.50 | 23.66 | 26.99 | 216.87 | 539.03 | 755.90 | 26.99 | 42.54 | 50.38 | 0.71 | 40.39 | 74.01 |
| TW | 2.65*** | 1.01*** | 0.01 | 1.1-4.8 | 2.53 | 0.05 | 0.15 | 3.74 | 0.01 | 0.33 | 0.34 | 3.74 | 22.77 | 23.08 | 0.97 | 1.17 | 46.29 |
| GFY | 0.53*** | 0.26*** | 0.01 | 0.21-4.8 | 1.61 | 0.05 | 0.14 | 5.45 | 0.01 | 0.75 | 0.76 | 5.45 | 53.78 | 54.06 | 0.99 | 1.78 | 110.23 |
| DMY | 0.02** | 0.06*** | 0.00 | 0.08-0.98 | 0.29 | 0.03 | 0.09 | 19.33 | 0.00 | 0.02 | 0.02 | 19.33 | 48.40 | 52.09 | 0.86 | 0.27 | 92.62 |

[@]Value under parenthesis represents degree of freedom; **Significant at 1% level of significance; ***Significant at 0.1% level of significance. [+]Z,: Zinc content; Cu, Copper content; Fe, Iron content; Mn, Magnese content; PH, Plant height (cm); NL, Number of leaves; LL, Leaf length (cm); LW, Leaf width (cm); TN, Tiller number; GN, Grain number; TW, Test weight (g), GFY, Green fodder yield (kg); DMY, Dry matter yield (kg); [$]SEm, Standard error of mean; CV, Coefficient of variation; $V_e$, Environmental variance; $V_g$, Genotypic variance; $V_p$, Phenotypic variance; ECV, Environmental coefficient of variance; GCV, Genotypic coefficient of variance; PCV, Phenotypic coefficient of variance; $H^2b$, Heritability (broad sense); GA, Genetic advance; GA(m), Genetic advance as percentage of mean.

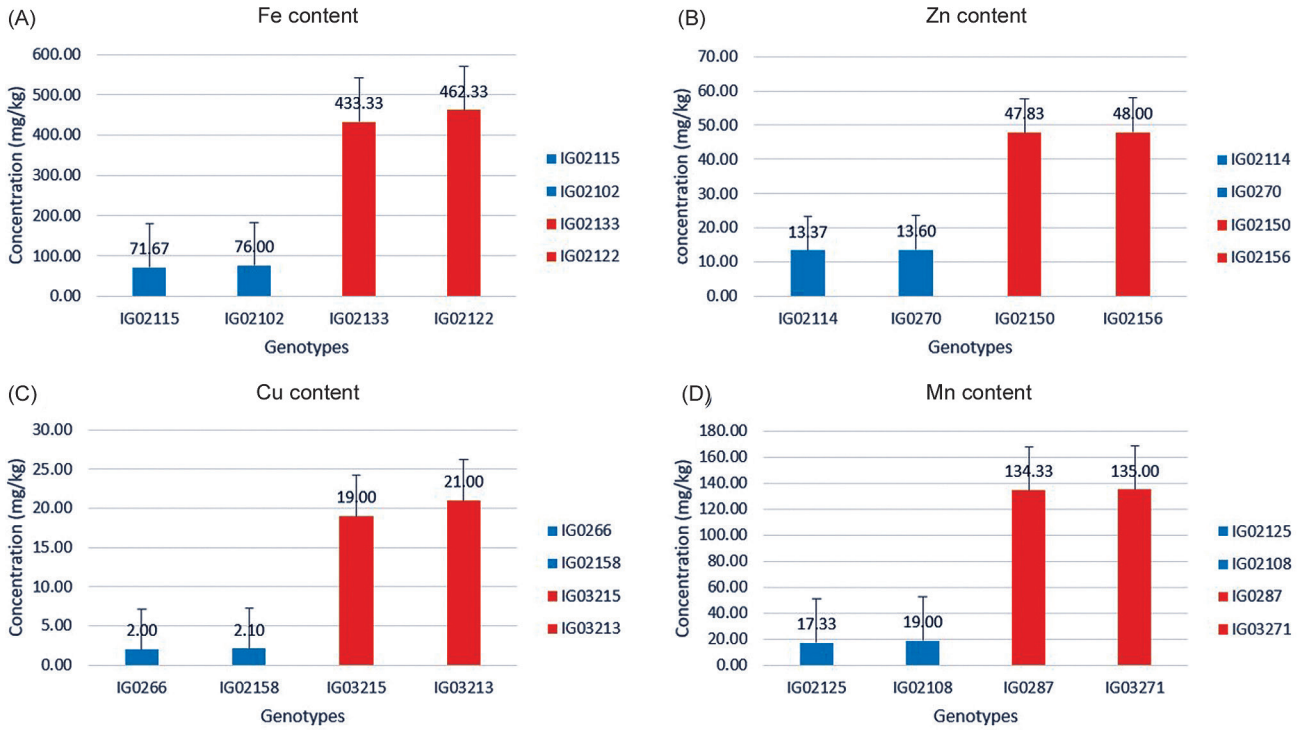


Fig. 1 Representation of two genotypes each in lowest and highest for (A) Fe; (B) Zn; (C) Cu and (D) Mn micronutrient content in fodder oat genotypes.

developing stable genotypes for these traits.

*Association Analysis*: Traits under study might show the correlation among them due to either linkage between the genes or pleiotropic effect and need specific care while doing selection for desirable traits (Sharma *et al.* 2022). In this study, Cu content had significant negative association with Mn content and positive with LL and LW at both genotypic and phenotypic level (Table 3). Therefore, selection for high Cu content in fodder type oats would decrease the Mn content and *vice-versa*. However, all other micronutrients had non-significant associations with each other in both positive and negative directions. Hence, selection for other micronutrients could be done simultaneously without any penalty. The GN had significant negative association with TW but significant positive correlation with GFY and DMY. Likewise, TW had significant negative association with TN but GFY had high significant positive association with DMY. However, GFY was positively correlated with most of the studied traits except NL and stem girth in oat accessions (Krishna *et al.* 2014).

*Principal component analysis*: It separated the total genetic variation among oat genotypes into five main components based on eigenvalues (≥1) and covered 59.09% of the total genetic variation (Supplementary Table 2). First principal component (PC1) covered the maximum proportion (17.13%) of the total genetic variation followed by PC2 (13.44%), PC3 (11.87%), PC4 (8.71%) and PC5 (7.94%). Morphological traits such as GFY, DMY and GN contributed significantly in PC1 but in negative direction. In PC2, GFY and DMY contributed in positive direction but LW and NL in negative direction. Mn content contributed positively, however Cu content, PL and TW played significant role in opposite direction in PC3. Likewise, traits such as Fe content and PH in PC4; TW and Zn content in PC5 contributed significantly in the total genetic variation.

Biplot denotes the distribution of accessions, observable parameters and association between them employing PCA scores on the X and Y axis (Fig. 2). The genotype-by-trait biplot also represents the graphical comparison between genotypes based on yield and associated traits. In this study, biplot had relatively longest vector for GFY, DMY, GN, LW and NL suggesting that these traits had contributed large dissimilarity among the genotypes, whereas Fe, Mn, Zn, Cu content, PH, LL and TW had lower vector length signifying their least discriminating ability. The GFY had acute (<90°C) angle with DMY, LL and GN indicating the positive correlation. Mineral contents, except Mn, had right (90°C) angle or slight obtuse (>90°C) angle with GFY and DMY highlighted that these traits did not have high correlation with forage yield. Biplot also revealed that genotypes IG02151, IG0259, IG02158, IG02149, IG02157 and IG0272 had contributed positively using TW and Fe content in PC1 whereas accessions such as IG0294, IG0277, IG02116, IG0270 and IG0298 had played significant role using GFY and DMY in PC2. Previously, Iannucci *et al.* (2011) reported 6 PCs covering 81% and Ihsan *et al.* (2021) reported four PCs having 57.60% of the total genetic variation in oat accessions based on morphological traits.

*Cluster analysis*: Circular dendrogram revealed six major groups and several sub-groups based on the

Table 3 Correlation coefficient of 13 traits including 4 forage micronutrient contents of 150 oat genotypes at phenotypic ($r_P$) and genotypic ($r_G$) level

| Parameter | Forage micronutrient and fodder related traits[#] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Correlation | Cu | Fe | Mn | PH | NL | LL | LW | TN | GN | TW | GFY | DMY |
| Zn | $r_P$ | 0.025$^{NS}$ | -0.103$^{*}$ | 0.065$^{NS}$ | -0.039$^{NS}$ | 0.093$^{*}$ | 0.002$^{NS}$ | 0.032$^{NS}$ | -0.027$^{NS}$ | -0.011$^{NS}$ | -0.165** | -0.078$^{NS}$ | -0.075$^{NS}$ |
| | $r_G$ | 0.025$^{NS}$ | -0.103$^{NS}$ | 0.065$^{NS}$ | -0.045$^{NS}$ | 0.136$^{NS}$ | 0.007$^{NS}$ | 0.054$^{NS}$ | -0.041$^{NS}$ | -0.016$^{NS}$ | -0.167* | -0.08$^{NS}$ | -0.080$^{NS}$ |
| Cu | $r_P$ | | 0.001$^{NS}$ | -0.220$^{**}$ | 0.045$^{NS}$ | 0.045$^{NS}$ | 0.175** | 0.117* | 0.127$^{**}$ | 0.016$^{NS}$ | 0.14** | -0.050$^{NS}$ | -0.075$^{NS}$ |
| | $r_G$ | | -0.0003$^{NS}$ | -0.223$^{**}$ | 0.051$^{NS}$ | 0.071$^{NS}$ | 0.243** | 0.203* | 0.153$^{NS}$ | 0.023$^{NS}$ | 0.143$^{NS}$ | -0.050$^{NS}$ | -0.084$^{NS}$ |
| Fe | $r_P$ | | | -0.005$^{NS}$ | 0.047$^{NS}$ | -0.040$^{NS}$ | 0.013$^{NS}$ | -0.093$^{*}$ | -0.057$^{NS}$ | -0.020$^{NS}$ | -0.089$^{NS}$ | -0.023$^{NS}$ | -0.021$^{NS}$ |
| | $r_G$ | | | -0.006$^{NS}$ | 0.056$^{NS}$ | -0.060$^{NS}$ | 0.027$^{NS}$ | -0.149$^{NS}$ | -0.069$^{NS}$ | -0.024$^{NS}$ | -0.091$^{NS}$ | -0.023$^{NS}$ | -0.022$^{NS}$ |
| Mn | $r_P$ | | | | -0.044$^{NS}$ | 0.071$^{NS}$ | -0.068$^{N}$ | 0.039$^{NS}$ | -0.039$^{NS}$ | 0.102$^{*}$ | -0.141** | 0.086$^{NS}$ | -0.010$^{NS}$ |
| | $r_G$ | | | | -0.052$^{NS}$ | 0.109$^{NS}$ | -0.099$^{NS}$ | 0.061$^{NS}$ | -0.051$^{NS}$ | 0.121$^{NS}$ | -0.144$^{NS}$ | 0.086$^{NS}$ | -0.009$^{NS}$ |
| PH | $r_P$ | | | | | 0.098* | 0.076$^{NS}$ | 0.136** | 0.105$^{*}$ | 0.105$^{*}$ | -0.032$^{NS}$ | 0.038$^{NS}$ | 0.043$^{NS}$ |
| | $r_G$ | | | | | 0.172* | 0.082$^{NS}$ | 0.178* | 0.136$^{NS}$ | 0.118$^{NS}$ | -0.038$^{NS}$ | 0.038 $^{NS}$ | 0.053$^{NS}$ |
| NL | $r_P$ | | | | | | -0.100$^{*}$ | 0.270** | 0.086$^{NS}$ | 0.154** | -0.092$^{NS}$ | 0.070$^{NS}$ | -0.027$^{NS}$ |
| | $r_G$ | | | | | | -0.121$^{NS}$ | 0.58** | 0.216$^{**}$ | 0.256** | -0.131$^{NS}$ | 0.111$^{NS}$ | -0.018$^{NS}$ |
| LL | $r_P$ | | | | | | | 0.109$^{*}$ | 0.081$^{NS}$ | 0.110* | 0.105$^{*}$ | 0.226** | 0.132** |
| | $r_G$ | | | | | | | 0.087$^{NS}$ | 0.101$^{NS}$ | 0.206* | 0.150$^{NS}$ | 0.317** | 0.177* |
| LW | $r_P$ | | | | | | | | 0.130** | 0.122** | -0.045$^{NS}$ | -0.019$^{NS}$ | -0.074$^{NS}$ |
| | $r_G$ | | | | | | | | 0.215** | 0.220** | -0.060$^{NS}$ | -0.030$^{NS}$ | -0.083$^{NS}$ |
| TN | $r_P$ | | | | | | | | | 0.128** | -0.132** | 0.046$^{NS}$ | 0.019$^{NS}$ |
| | $r_G$ | | | | | | | | | 0.152$^{NS}$ | -0.167* | 0.049$^{NS}$ | 0.016$^{NS}$ |
| GN | $r_P$ | | | | | | | | | | -0.153** | 0.369** | 0.254** |
| | $r_G$ | | | | | | | | | | -0.184* | 0.445** | 0.323** |
| TW | $r_P$ | | | | | | | | | | | -0.093* | -0.033$^{NS}$ |
| | $r_G$ | | | | | | | | | | | -0.100$^{NS}$ | -0.038$^{NS}$ |
| GFY | $r_P$ | | | | | | | | | | | | 0.72** |
| | $r_G$ | | | | | | | | | | | | 0.774 ** |

*Significant at 5% and **Significant at 1% level of significance; NS, Non-significant; Zn, Zinc content; Cu, Copper content; Fe, Iron content; Mn, Magnese content; PH, Plant height (cm); NL, Number of leaves; LL, Leaf length (cm); LW, Leaf width (cm); TN, Tiller number; GN, Grain number; TW, Test weight (g), GFY, Green fodder yield (kg); DMY, Dry matter yield (kg).

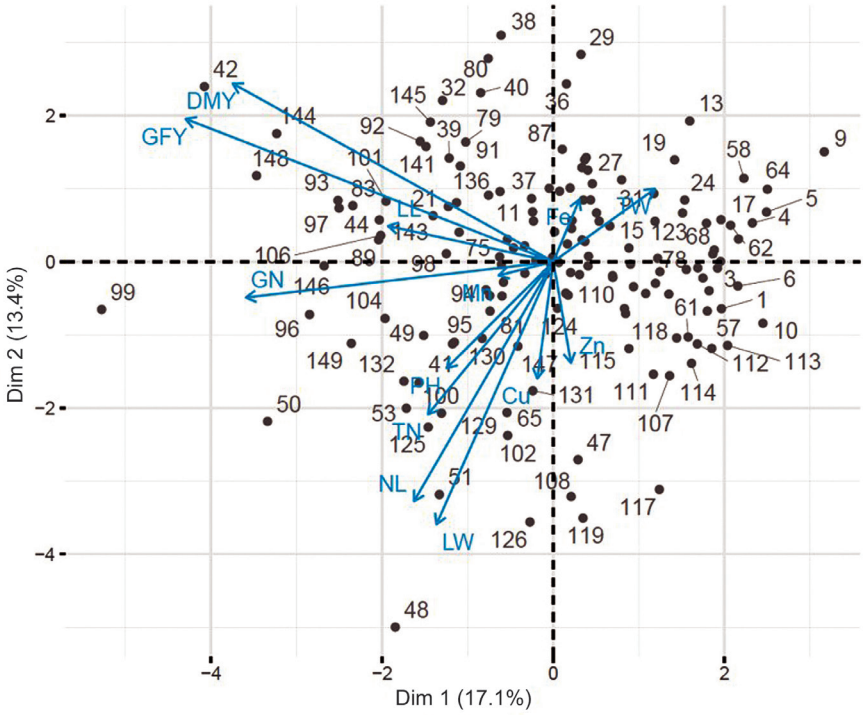


Fig. 2 Principal component analysis bi-plot shows eigenvectors of each of the 13 traits including forage micronutrients and fodder related traits (blue arrows) and PCA scores for all combined phenotypes of 150 genotypes (denoted by black dots).
Note: The magnitude of each vector shows the weightage contribution of each trait to each PC. Arrows pointing in same direction indicate positive correlation between them, however arrows pointing opposite direction indicate negative correlation between variables and arrows at right angles indicate no or low correlation. Dim1 and Dim2 represent PC1 and PC2, respectively.

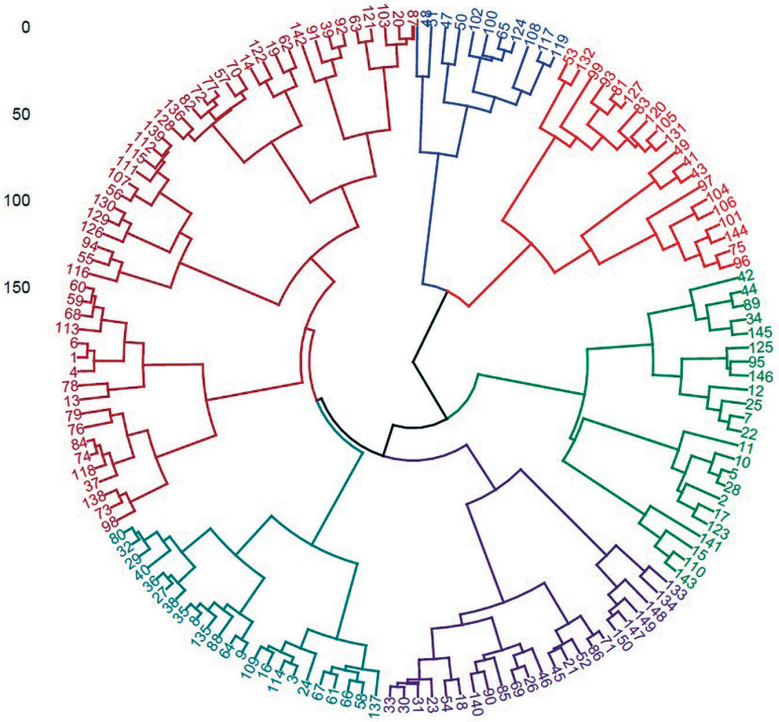

Fig. 3 Circular dendrogram and complete hierarchical clustering of 150 oat genotypes based on Mahalanobis distances.
Note: Genotypes with same colour are grouped in same cluster and *vice-versa*.

micronutrients, fodder yield and associated traits (Fig. 3). The study highlighted that cluster 4 had the maximum number of accessions (40) followed by cluster 1 (38), cluster 5 (26), cluster 3 (25), cluster 6 (12), and cluster 2 (9). The nature of clustering was non-overlapping where each genotype was placed only in a single cluster. Most of the genotypes accessed from the USA and Sweden were bunched into different clusters indicated that accessions collected from the same source had more dissimilarity for the studied traits. It also highlighted that genotypes collected from the same source might have different ancestral lineage or collected to a country from different source of origin. Therefore, selection of suitable parents must be based on genetic dissimilarity rather than geographical diversity for crop improvement programmes. Mahalanobis distance was maximum between cluster 4 and 5 (10.45) followed by cluster 3 and 5 (9.50). The least Mahalanobis distance was found between cluster 1 and 4 (7.88). The intra-cluster distance was maximum for cluster 5 (9.02) followed by cluster 3 (8.30), cluster 6 (7.77) and cluster 1 (7.12). The inter-cluster distance was higher than the intra-cluster distance indicating the presence of high genetic diversity among the lines belongs to different cluster rather than genotypes from the same cluster. Besides, cluster 5 and 4 had the highest cluster mean. Traits such as PH, NL, LW, TN, GN, GFY and DMY contributed significantly in cluster 5 for higher cluster mean whereas Cu, Fe, Mn content, LL, TW and GFY contributed in cluster 4. Therefore, genotypes from these two clusters can be used as parents for the future breeding programmes. Previously, 50 oat genotypes were grouped into 10 clusters (Krishna *et al.* 2014) whereas another set of 50 oat accessions were categorized into 6 clusters (Kumari and Jindal 2019) based on morphological traits.

Biofortified fodder varieties would not only fulfill the nutritional demand of livestock but also would reduce the feeding cost to the livestock keepers. Developing the biofortified fodder varieties needs the suitable donor lines having high micronutrient content at 50% flowering stage where crop is used as livestock feed. The selected oat genotypes having high

Fe (IG02133, IG02122), Zn (IG02150, IG02156), Mn (IG0287, IG03271), and Cu (IG03215, IG03213) content could be used as donor in backcross breeding programs to enrich the micronutrient status of high yielding but low-micronutrient varieties or using them in hybridization programs for broadening the genetic base of existing lines.

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