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Biochemical delineation of oat (*Avena sativa*) accessions for nutritional improvement

RUKOO CHAWLA[1,2]*, MINAKSHI JATTAN[1], D S PHOGAT[1], NISHA KUMARI[1], SANDEEP KUMAR[1], AMIT SHARMA[1], DEEKSHA CHAUHAN[2] and NEELAM KUMARI MANDAL[3]

CCS Haryana Agricultural University, Hisar, Haryana 125 004, India

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ABSTRACT

Oat (*Avena sativa* L.) is a unique multifaceted crop used for fodder and grain purpose. It's grain has tremendous potential to offer health benefits, especially with the heightened emphasis on nutrition and food security. With this aim, quality traits were investigated among 62 oat genotypes, demonstrating significant variation. The biochemical analysis was conducted in laboratory of department of Genetics and Plant Breeding of CCS Haryana Agricultural University, Hisar during 2019–21. Quality parameters depicted a wide range for seed crude protein (8.16–19.18%), forage crude protein (5.17–11.42%), phenol (0.61–1.22%), beta-glucan content (0.32–7.55%), total soluble sugar (4.90–8.49%), reducing sugar (1.07–4.28%) and non-reducing sugar (2.02–6.38%). The current research covered wide and powerful analytical approaches that helped to underpin the selection of the most promising genotypes and evaluated the contribution of different traits to heterogeneity. Furthermore, non-reducing sugar, reducing sugar and seed crude protein were emerged to be the major contributors of PC1, PC2 and PC3, respectively. The genotypes GP 492, HFO 1107, HFO 1003, HFO 1016, OS 403, HFO1105 and HFO 806 were the best performing based on quality parameters. Promiscuous genotypes can serve as pioneers in oat improvement programs, enabling the enhancement of nutritional value. These insights expand the prospects for the food industry and hence appraise the significance of oats among other cereals.

Keywords: Cluster analysis, Diversity, Oat, Principal component analysis, Quality

The world population is projected to reach 9.7 billion in 2050 (World Health Organization 2021). Due to inadequate quantity or subpar food quality, malnutrition has been documented in approximately 815 million people worldwide. Subsequently, it is imperative to put forth substantial efforts in boosting both the quantity and quality of food (Sheoran *et al.* 2022). The ideal approach for breeders is to avail the untapped and extensive germplasm collections, which may be an effective tool to broaden the genetic pool. Oat (*Avena sativa* L.) covers a worldwide area of 9.4 million hectares with a production of 24.3 million metric tonnes (USDA August 2022). It has good digestibility, higher nutritional value and a higher globulin fraction of storage protein (Klose and Arendt 2012, Rodehutscord *et al.* 2016). Furthermore, it is rich in minerals, antioxidants and total dietary fibre (Oliver *et al.* 2010). Beta-glucan in oat makes it a healthy substitute that lowers low-density lipoprotein, serum cholesterol and ultimately, the likelihood of heart disease and diabetes (Tiwari and Cummins 2011, Ho *et al.* 2016, Pino *et al.* 2021). In addition, oat avenins lack any of the recognized celiac disease epitopes found in wheat, barley or rye gluten (Storsrud *et al.* 2003, Smulders *et al.* 2018). These quality aspects of oat have accentuated its capability of having multi-functional grain qualities among other cereals. Oats are not a significant source of sugars compared to other grains (Kaur *et al.* 2019). In addition, the exact amount of reducing sugars in oats can vary depending on the variety and growing conditions. A relatively small amount of reducing sugar is present in oat. Oats contain non-reducing sugars such as sucrose, which is the most abundant sugar in oats (USDA Food Data 2021).

Furthermore, the dual nature of oat also facilitates feed uses (Chawla *et al.* 2022). Due to its fast regeneration tendency, multi-cut character and high nutritional content, oat makes an excellent fodder crop (Ross *et al.* 2004). The biochemical documentation is a practical approach for generating core collections with comprehensive coverage and formulating breeding schemes. In this study, multivariate analysis (PCA and clustering) was executed, along with other

[1]CCS Haryana Agricultural University, Hisar, Haryana; [2]Maharana Pratap University of Agriculture and Technology, Udaipur Rajasthan; [3]Government (PG) College, Panchkula Haryana. *Corresponding author email: rukoochawlamanjul@gmail.com

statistical techniques, to facilitate the selection of superior heterotic parents to utilize diverse improved cultivars (Sharma *et al.* 2023).

MATERIALS AND METHODS

The present experiments were conducted in the laboratory of Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (29°10'N and 75°44'E and 228 m amsl) Haryana during 2019–21. Sixty-two oat genotypes (Supplementary Table 1) were evaluated for important quality traits. The biochemical traits including seed crude protein, forage crude protein (Micro-Kjeldahl method; Horneck and Miller 1997), beta-glucan (β-Glucan Megazyme kit), phenol content (Hillis and Swain 1959), total soluble sugar (Dubois *et al.* 1956), reducing sugar (Nelson 1944, Somogyi 1945) and non-reducing sugar (calculated by subtracting total soluble sugar with reducing sugar) were investigated. All the figures were drawn using R software. A hybrid approach, named hierarchical k-means clustering, was used, which is an improvement over k-means clustering. Hierarchical clustering was performed initially (Supplementary Fig 1) and then the k-means method was applied to enhance the original partitioning created under this. Hence, the genotype clustering is slightly different from the final cluster plot compared with the dendrogram.

For crude protein analysis, in a 100 ml Micro-Kjeldahl's digestion flask, 100 mg of dried material was placed. One gram of a K_2SO_4:$CuSO_4$ (9:1) combination was added, followed by 10 ml of concentrated sulfuric acid. Then, flasks were gently heated while being kept on the hot plate in the digesting chamber until the solution became translucent and took on a blue-green hue. After the flask's contents had cooled, distilled water was added to make up the capacity before being transferring to a 100 ml volumetric flask. After adding 10 ml of 40% NaOH, 10 ml of the aliquot of the above-prepared solution was collected for distillation. The ammonia was absorbed in a flask containing 10 ml of N/100 sulphuric acid and one or two drops of methyl red indicator. By titrating it with N/100 sodium hydroxide and a blank sample, a blank was also digested and distilled to determine the quantity of ammonia absorbed by N/100 sulphuric acid. For total soluble sugar, one millilitre of the diluted sugar extract (1 ml of sugar extract + 9 ml of distilled water) was placed in a test tube. Two millilitres of the 2% phenol solution and five millilitres of the concentrated sulphuric acid (H_2SO_4) were then added. Test tubes were gently oscillated after each reagent addition, and they were then allowed to cool for 30 minutes. The produced solution's absorbance was measured at 490 nm using a UV-Vis spectrophotometer (Systronics India). The concentration of total sugars was estimated from the standard curve of glucose, which was constructed concurrently with the experiment.

Following the addition of one ml of the freshly made sample extract and one ml of alkaline copper reagent in the test tube, an assessment of the quantity of reducing sugars was done. The mouth tubes were placed in a boiling water bath with a cover on them for 20 minutes, after which tubes were allowed to cool in an ice bath. After cooling, one ml of the arsenomolybdate reagent was added, and the absorbance at 520 nm was recorded. For the analysis of the blank reagent, one ml of distilled water was used in place of the sample extract. The standard curve made from glucose solution (5–50ug) was used to calculate the amount of reducing sugar. Non-reducing sugar is computed by deducting the amount of total soluble sugar from the amount of reducing sugar. Furthermore, to estimate the total phenol (TP) levels, one millilitre of the produced extract was placed in a test tube, and the solution was carefully mixed before being diluted to 7.5 ml with distilled water. Following the addition of 0.5 ml of diluted Folin-Ciocalteau reagent, one ml of saturated sodium carbonate, together with 10 ml of distilled water, were added after three to five minutes. The UV-Vis spectrophotometer (Systronics, India) was used to measure the absorbance at 725 nm after the tube had stood for an hour. Distilled water was used in lieu of the sample extract as a reagent blank. For beta glucan, extraction method was measured by using a mixed-linkage β-Glucan Megazyme kit. Samples were hydrated and suspended in a buffer solution with a *p*H of 6.5. Following an incubation with a pure lichenase enzyme, contents were filtered. A portion of the filtrate was fully hydrolyzed using pure -glucosidase. A glucose oxidase/peroxidase reagent was used to measure the D-glucose that was generated.

β-Glucan (% w/w)

$$= \Delta A \times F \times FV/0.1 \times 1/1000 \times 100/W \times 162/180 \times D$$

$$= \Delta A \times F/W \times FV \times D \times 0.9$$

where ΔA, Absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance; F, Factor for the conversion of absorbance values to μg of glucose; FV, Final volume; 0.1, Volume of sample analyzed; 1/100, Conversion from μg to mg; 100/W, Factor to express β-glucan content as a percentage of sample weight; W, The weight in mg ("as is" basis) of the sample analyzed; 162/180, factor to convert from free D-glucose, as determined, to anhydro-D-glucose, as it occurs in β-glucan; D, Further dilution prior to incubation with β-glucosidase (if required).

B-glucan % w/w (dry wt. basis) = β-glucan % w/w × 100/100 – moisture content (% w/w)

RESULTS AND DISCUSSION

Variability in quality traits for nutritional enhancement: Components are separated and refined from oat grain during the development of nutritional supplements and marketed in therapeutic forms that are not often connected with food goods. Many new and future uses of this crop will be supported by tailoring the nutritional and functional properties of the oat grain (Sterna *et al.* 2016). Seven quality traits were estimated, i.e. seed crude protein (SCP), forage crude protein (FCP), phenol content, beta-glucan, total soluble sugar (TSS), reducing sugar (RS) and non-reducing sugar (NRS). Based on these qualitative characters, 10 superior performing genotypes selected from

62 oat genotypes (Table 1). The present study illustrated a significant amount of variability for seed crude protein (8.16–19.18% with mean of 12.31%) and forage crude protein (5.17–11.42% with an average of 5.17%) among the oat genotypes evaluated. Beta-glucan is a more valuable trait serving as a functional food ingredient and has a crucial role in the medical industry. In the current research, the genotypes exhibited a remarkable variability (0.32–7.55%) for beta-glucan. However, the phenol content was comparatively less prominent and varied (0.61–1.22%) among the genotypes. Furthermore, TSS, RS and NRS ranged from 4.90–8.49%, 1.07–4.28% and 2.02–6.38%, respectively.

According to Prates and Yu (2017), oat has the highest protein content among cereals and it is reported that different oat cultivars used for milling and feeding might have total sugar contents ranging from 1.17–3.8%. As per a report, oat grains contain significantly more and superior quality protein than wheat (Rodehutscord *et al.* 2016). Scrutinizing a diverse and large number of accessions for quality traits will help select the best performing genotypes. These can be incorporated in human meals for nutritional enhancement. Markovic *et al.* (2017) found a similar range of variability (3.15–7.28%) in β-glucan content among oat varieties. Emmons *et al.* (2001) investigated how site affected the phenolic content and antioxidant potential of oat groats and discovered that significant cultivar × location interactions existed among the genotypes for all the phenolic compounds.

Cluster analysis: Based on 7 quality traits, four cluster groups were formed from 62 oat genotypes. Cluster 2 was the largest, with the maximum number of genotypes (18), followed by cluster 1 (16 genotypes). Clusters 3 and 4 consisted of 14 genotypes each (Fig 1) (Supplementary Table 2). The maximum inter-cluster distance existed between cluster 3 and cluster 4 (4.01) followed by clusters 2 and 4 (3.95) and clusters 1 and 4 (3.90). Cluster 4 and cluster 1 had the maximum (3.21) and minimum (2.59) intra-cluster distance, respectively (Supplementary Table 3). The clustering and association patterns were validated using a conceptual model in the form of a heat map (Fig 2). The heat map depicts close association of SCP with FCP, green fodder yield (GFY) with seed yield (SY), TSS with NRS. The inter-cluster distance between different clusters was more and the intra-cluster distance of the respective cluster was less, which confirms a good clustering algorithm. Cluster 1 had genotypes with high RS (HFO1105, HFO1115, C3(UPO212). Cluster 2 composed of genotypes enriched with high TSS (OS 403, C2(JHO 851) and NRS (HFO 806, C2(JHO 851). Additionally, Cluster 3 had genotypes with high FCP (HFO 1016, HFO 1108, HFO 1106). Cluster 4 had genotypes with higher beta glucan (GP 492). The genotypes can be selected from diverse clusters performing well for the quality traits for crop improvement programmes. For instance, the most diverse cluster 3 (JO-1 and KENT) and 4 (GP 492) both had few genotypes rich in beta glucan. Thus, breeders should involve genotypes from these two clusters in hybridization programmes which are diverse yet

Table 1 Top 10 best performing oat genotypes for different quality traits

Beta-glucan (%)		Phenol (%)		Seed crude protein (%)		Forage crude protein (%)	
GP 492	7.55	HFO 1107	1.22	HFO 1003	19.18	HFO 1016	11.42
HFO 1113	6.68	HFO 1106	1.18	OL 1861	17.95	HFO 1108	10.83
JO-1	6.24	OL 125	1.13	HFO 915	17.14	HFO 1106	10.46
KENT	6.04	HFO 818	1.12	HFO 1109	16.32	HJ 8	10.21
HFO 902	5.95	HFO1123	1.12	HFO 1118	16.32	HFO 1122	9.79
HFO 1109	5.65	RO-11-2-2	1.12	HFO 1114	15.51	GP 68	9.53
HFO 903	5.58	PLP-1	1.12	JHO 2006-1	15.09	HFO 529	9.38
NDO-1	5.48	JO-1	1.12	HFO 607	15.09	HFO 607	9.38
HFO 915	5.32	C2(JHO851)	1.09	HFO 818	14.69	HFO 903	9.38
RO-11-2-6	5.31	GP875	1.07	C3(UPO212)	14.69	OL 125	9.38
Total soluble sugar (%)		Reducing sugar (%)		Non-reducing sugar (%)			
OS 403	8.49	HFO1105	4.28	HFO 806	6.38		
C2(JHO851)	8.41	HFO1115	3.67	C2(JHO851)	6.29		
HFO 607	8.23	OL1766-2	3.52	OS 403	6.26		
OL1766-2	8.21	C3(UPO212)	3.48	HFO 1123	6.22		
HFO1109	8.16	HFO 529	3.45	HFO 607	6.14		
HFO 529	8.12	GP192	3.42	HFO 903	5.82		
JHO 822	7.98	HFO1117	3.39	HFO 707	5.48		
HFO 707	7.82	RO-11-2-2	3.24	GP 781	5.33		
HFO 806	7.76	HFO1113	3.23	HFO 1109	5.18		
GP 192	7.56	HFO1111	3.19	HFO 1121	5.12		

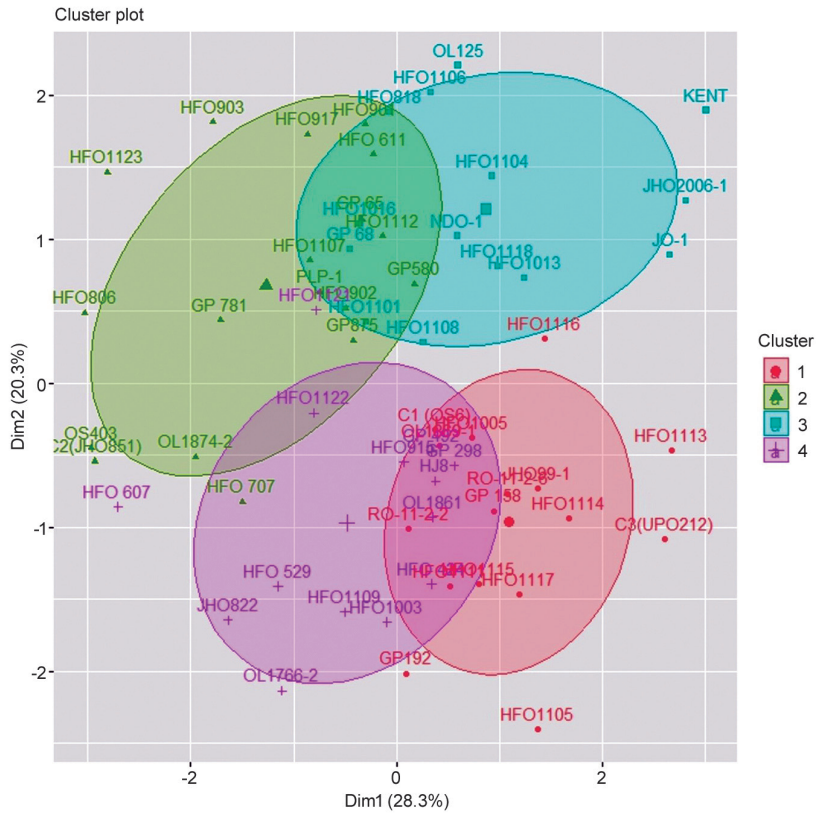

Fig 1 Final partitioning obtained after applying k-means algorithm to the initial hierarchical clustering. Sixty-two oat genotypes were grouped into four clusters.

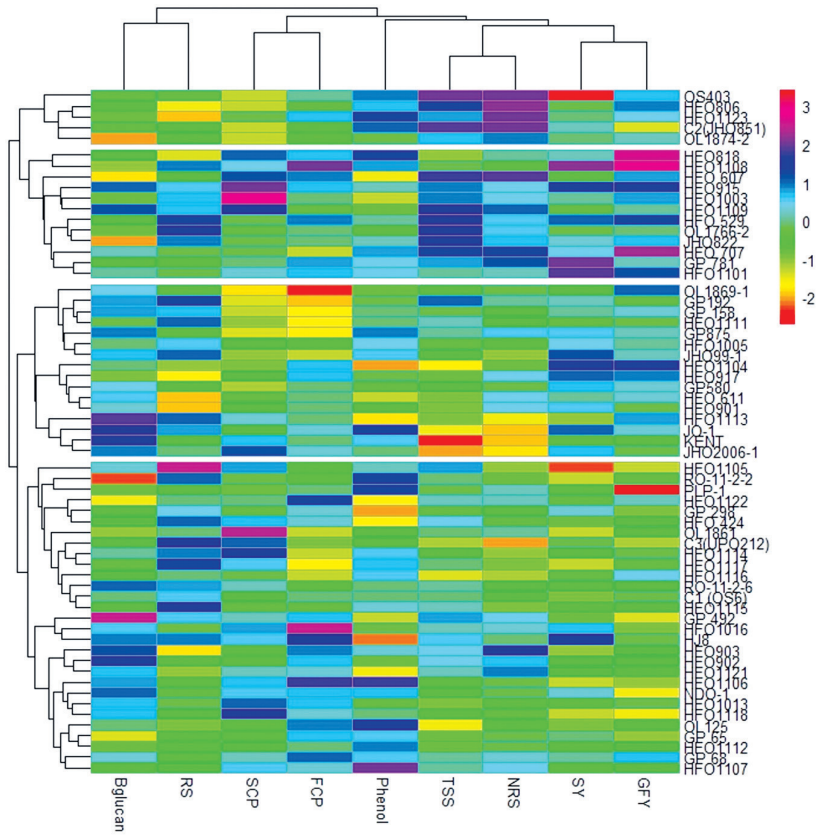

Fig 2 Heat map using R studio software, after scrutinizing the cluster plot, biplot and heat map.

enriched with high quality traits.

Principal component analysis (PCA): PCA is a technique for reducing the dimensionality and enhancing the data set's interpretability. This is achieved by introducing unique, uncorrelated variables that sequentially boost variance (Jolliffe and Cadima 2016). PCA was implemented for seven quality traits on 62 oat genotypes. The main principal components having eigen values greater than one were the most essential in capturing the patterns of variation among the variables (Table 2). These three main components contributed a cumulative variance of 68.1% (PC 1–28.3%; PC 2–20.3% and PC 3–19.5%). PC 1 accounts largely for NRS, TSS and beta glucan while PC 2 was impacted by RS, DF, TSS and FCP. It was further detected that PC 3 was loaded on SCP and FCP. Biplot analysis identified four groupings (Fig 3). The first group comprised of seed crude protein and reducing sugar, positively affected by the PC1 and negatively affected by PC2. Only the beta-glucan trait was present in the second group, which was related favourably to both PC 1 and PC 2. Furthermore, phenol, NRS and FCP were contained in the third group having a negative impact on PC 1 but a positive impact on PC 2. Lastly, the fourth group had TSS which negatively contributed to PC1 and PC 2.

A biplot was drawn combining both score plot (portraying PC score of each of 62 oat genotypes for all the 7 traits) and loading plot (depicting how the original variables in the form of a loading vector, contribute to creating the principal components) in one graph. Biplot visualization provides immense information and different interpretations can be made. The more parallel a variable vector is to a principal component axis, the prominent it contributes only to that PC. The longer vectors in the biplot, i.e. TSS, NRS, RS and FCP, signified greater variability for these traits by both the principal components (PC 1 and PC 2). The angle between the vectors may also be used to examine the correlation between attributes; the greater the acute angle, greater will be the positive correlation and the greater the obtuse angle, more influential will be the negative correlation (Chawla *et al.* 2021).

The genotypes, viz. HFO 1106 (high phenol and FCP), C2 (JHO 851) (high TSS,

Table 2 Principal components extracted for different quality traits and factor variable correlations

Parameter	Principal component		
	PC 1	PC 2	PC 3
Eigen value	1.407	1.193	1.170
Proportion of variance	0.283	0.203	0.195
Cumulative proportion (%)	28.3	48.6	68.1
Trait	*Factor loadings/eigen vectors*		
Seed crude protein	0.198	-0.090	0.618
Fodder crude protein	-0.133	0.409	0.580
Beta-glucan	0.323	0.161	0.148
Phenol	-0.080	0.243	-0.479
Total soluble sugar	-0.522	-0.507	0.137
Reducing sugar	0.305	-0.694	0.031
Non-reducing sugar	-0.683	0.020	0.103

NRS and phenol), HFO 607 (high SCP, FCP, NRS and TSS), HFO 529 (high FCP, TSS and RS), HFO 1109 (high SCP, NRS and beta glucan), JO-1 (high phenol and beta-glucan) were found most promising. Evaluation of diversity among oat genotypes led to identification of potential genotypes for high quality. Such genotypes are likely to be used to broaden the gene pool to incorporate these genotypes in oat improvement programmes. The potential role of various quality traits can be studied through multivariate analysis. Studying biochemical parameters can provide information about the functional properties of oats and how they can be used in various food applications. Additionally, screening of genotypes based on critical nutritional parameters will be effective in targeting nutritional improvement along with providing benefits to the food industry and medical sector. This information can be used to develop new food products and formulations that make use of the unique properties of oats, and to promote the consumption of oats as part of a healthy diet.

Fig 3 Biplot (score plot + loading plot).

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