Cluster Analysis in Fodder Oats (*Avena sativa* L.)

Abstract

Oats is an important crop used globally for food and fodder, holding significant economic value. In the context of livestock farming, fodder oats (*Avena sativa* L.) is an important crop during winter season. A comprehensive analysis of thirty oat genotypes was conducted at The Regional Agricultural Research Station at Ambalavayal in Wayanadan Eastern Plateaux of Kerala to identify superior genotypes that could increase forage production and improve nutritional quality during the Rabi season of 2022-23. A cluster analysis using Mahalanobis D2 statistics was performed employing the Tocher method within the Indostat software, involving eleven morphological and six nutritional traits. The thirty genotypes were categorized into seven clusters based on their D^2 values using the Euclidean method. Cluster I consisted of 14 genotypes (OL-1942, OL-1944, OL-1980, OL-15, OL-212, OL-11, OL-1952, OL-1874-1, OL-1975-2, OL-1976-1, OL-12, OL-1967, AVT-1, OL-13), followed by Cluster II with 8 genotypes (OL-10, OL-2000, OL-1977, OL-1964, OL-1988, OL-1896, OL-1802, OL-1974). Cluster IV comprised 3 genotypes (OL-1937, OL-1963, OL-125), Cluster III included two genotypes (OL-9, JHO-822), and Cluster V(OL-1931-1), VI(OL-1969), and VII(OL-1949) each had one genotype. The inter-cluster D^2 values were found to be higher than the intra-cluster D2 values implying that there is a substantial amount of diversity among the genotypes under study with respect to the considered characters. The highest intra-cluster distance was observed in Cluster IV (42.81), followed by Cluster II, Cluster I, and Cluster III. The maximum inter-cluster D2 values were observed between Clusters IV and VII (102.31), and the minimum was observed between Clusters II and V (45.44). Based on the cluster mean, Cluster III was observed to be a significant contributor of days to first and 50% flowering, days to maturity, crude fibre content, total phenolic and antioxidant content. Cluster IV was a potential contributor to green fodder yield, dry matter yield, leaf and stem dry weight, plant height, and phytate content. Cluster V was associated with the number of tillers and crude protein content. Cluster VI was related to the number of leaves and condensed tannin content. Cluster VII was pertaining to the leaf-stem ratio.

Keywords: Clusters, fodder, oats, genotypes.

INTRODUCTION

Genetic diversity is an essential foundation for any crop improvement initiative. However, this valuable genetic variability is diminishing rapidly due to human development and the constant utilization of this diversity in ongoing crop improvement programs.

Oat (Avena sativa L.) is the most important cereal (graminaceous) forage crop grown during the winter season, which is used as food, feed and fodder. Oat fodder is mostly fed as green and surplus is converted into silage or hay for further use during the fodder deficit period (Suttie and Reynolds, 2004). It is a preferred feed of all the animals and its straw is soft and superior to wheat and barley. Oat is a self-pollinated allohexaploid crop (2n=6x=42) with basic chromosome number x=7 having a genomic constitution of AACCDD (Rines et al., 2006). The Avena genus is notably extensive and diverse, encompassing species of varying ploidy levels, including diploid, tetraploid, and hexaploid variants. Fodder oat is used as a multipurpose crop worldwide. They are usually winter sown, grazed prior to stem elongation and taken to maturity for use as feed and/or milling grains. Multiple cuts are usually taken, after which part or all of the crop may be saved for seed. Oat has adequate soluble carbohydrates and fibres (Peterson et al., 2005). Oat provides one of the richest

sources of the dietary soluble fibre beta-glucan, providing 5.0 g (oatmeal) to 7.2 g (oat bran) per 100 g serving (Glore et al., 1994). Nutrition experts believe that beta-glucan inhibits cholesterol buildup and hence helps in the prevention of heart disease (Whitehead et al., 2014). Oats have gained significance in India, serving as both a valuable source of fodder and grain for animal feed, especially for calves, young livestock, horses, poultry, and sheep. In dairy farming, oat fodder is indispensable as it can be provided fresh, with any surplus being preserved as silage or hay for use during periods of scarcity

About 10 million hectares of cultivated oat (Avena sativa L.) are planted each year, yielding approximately 23 million metric tonnes of grains worldwide (USDA, 2020-21). In India, oat is grown as a dual-purpose crop, covering approximately 0.1 million hectares and yielding 35-50 tonnes of green fodder per hectare (Anonymous, 2014). It is mainly grown during rabi season in many states across the country, including the North Western, southern and eastern states. The crop provides green fodder during the winter season in the Himalayan region, when green fodder is scarce which is rich in approximately 10-13% protein and 10-30% dry matter (Priyanka et al., 2021).

MATERIALS AND METHODS

The experiment was conducted during the 2022 Rabi season at the research farm, Regional Agricultural Research Station in Ambalavayal. The study was conducted in Randomized block design with three replications utilising a total of thirty genotypes of fodder oats sourced from Punjab Agricultural University and assessed their fodder yield and quality. The list of these thirty Avena sativa L. genotypes can be found in Table 1. Evaluation of these genotypes encompassed 17 different characteristics, in accordance with recommended agronomic practices. The crops were planted with a row-to-row distance of 30cm and a plant-to-plant distance of 10cm.

Observations were recorded from five randomly selected plants for each treatment within each replication at the time of harvest. The mean values of these five plants within each replication were utilized for subsequent statistical analysis. These observations were made during the milky stage of grain filling, focusing on 11 morphological attributes, includes days to first flowering, days to 50 per cent flowering, number of tillers per plant, number of leaves per plant, green fodder yield per plot (in kilograms), dry matter yield per plot (in kilograms), leaf dry weight per plant (in grams), stem dry weight per plant (in grams), plant height (in centimetres), days to maturity, and leaf-to-stem ratio.

Additionally, six nutritional characteristics were evaluated, including crude protein content (mg/g), crude fibre content (mg/g), total phenolic content [% gallic acid equivalents (GAE)], condensed tannin content (% of catechin), total antioxidant content (µg of ascorbic acid per milligram), and phytate content (mg/g). To assess the degree of divergence among different pairs of statistics, Mahalanobis' D^2 statistics, as proposed by Mahalanobis in 1936, were employed. The D^2 values and group constellations were computed using the method outlined by Rao in 1952. The genotypes were grouped into different clusters on the basis of Ward's minimum variance method.

RESULTS AND DISCUSSION

The analysis of variance showed highly significant differences within the population for all the 17 characters studied. This suggested that the genotypes under investigation consisted a sufficient amount of diversity and also indicated that this material was appropriate for the estimation of further analysis. The earlier workers Singh and Singh (2011); Shehzad et al. (2011); Bibi et al. (2012); Hisir et al.

(2012); Bind et al. (2016) also suggested a large and exploitable variation in different oat germplasm, it could be stated that there is ample scope of variation in these traits that could be utilized for improvement through selection for the traits investigated in the present material. On the basis of D^2 values, the 30 genotypes were grouped into seven clusters following Tocher's method. The I and II were the largest ones with 14 and 8 genotypes each, followed by IV, III, V, VI and VII with 3,2,1,1 and genotypes respectively. A significant amount of variability can be inferred from the pattern of group constellation (Table 2). This revealed that the pattern of clustering of genotypes did not depend upon their geographical origin. It means that the genotypes from the same geographical origin do not group together i. e. genetic constitution of the genotypes is dominant. A dendrogram generated in which seven clusters are clearly distinguished is shown for 30 genotypes in Fig. 2. Bahadur and Choubey (2008), Yadav et al. (2011), Ahmed et al. (2011), Krishna et al. (2014) and Kumar et al. (2016) suggested that the cluster with a higher number of lines had low genetic diversity among the lines and they were more closely related.

The average inter-cluster D^2 values, as well as the intra-cluster D^2 values for the seven clusters, are presented in Table 3. Notably, the inter-cluster D^2 values were found to be higher than the intra-cluster D^2 values. This observation indicates that there is a substantial amount of diversity among the genotypes under study with respect to the considered characters. In other words, there is significant variation between different clusters, highlighting the diversity present in the population.

The range of intra-cluster D^2 values displayed variability, with the highest recorded at 42.81 in cluster IV and the lowest at 19.82 in cluster III. Cluster IV exhibited the greatest intra-cluster distance, followed by cluster II at 35.71, cluster I at 30.71, and cluster III at 19.82. Notably, clusters V, VI, and VII all yielded intra-cluster D^2 values of zero. This was due to the presence of only a single genotype within each of these clusters, signalling a lack of diversity within these particular clusters.

The maximum inter-cluster D^2 values were observed between clusters IV and VII (102.31), followed by clusters I and VII (100.54), Cluster VI and VII (97.52), cluster IV and V (83.26), III and VI (77.57), III and IV (73.91), I and V (69.29), III and VII (68.79), V and VII (64.04), V and VI (63.84), II and IV (61.64), II and VII (60.83), II and I (58.14), III and I (57.85), II and VI (57.68), III and V (54.37, IV and VI (53.78), II and III (48.82) and I and VI (47.99). The minimum inter-cluster D2 value was found between clusters II and V which was 45.44. These values indicate the extent of dissimilarity between different pairs of clusters, with the highest dissimilarity observed between clusters IV and VII.

The dendrogram in Figure 2. illustrates the clustering pattern of 30 oat genotypes. This pattern was determined using Ward's minimum variance method in a non-hierarchical Euclidean cluster analysis.

The cluster means for the seventeen traits analyzed in the 30 fodder oats accessions revealed significant variations across the clusters, as illustrated in Table 4. The data highlights Cluster III as a key contributor to several traits, including days to first flowering (74.37), days to 50% flowering (84.78), days to maturity (124.26), crude fiber content (25.99), total phenolic content (204.12), and total antioxidant content (60.60). Cluster III stands out as particularly promising for selection due to its strong performance in these specific traits. Cluster IV also shows potential contributions, particularly in the traits of green fodder yield per plot (8.36), dry matter yield per plot (2.34), leaf dry weight per plant (7.51), stem dry weight per

plant (18.29), plant height (152.19), and phytate content (1.72). Cluster V is noteworthy for excelling in the number of tillers per plant (9.80) and crude protein content (16.16). Cluster VI exhibits the highest values for the traits related to the number of leaves per plant (21.03) and condensed tannin content (1.83), making it a unique cluster with strengths in these areas. Meanwhile, Cluster VII demonstrates the highest mean value for the leaf-to-stem ratio (0.75). In summary, each cluster showcases its own distinct strengths and characteristics, making them valuable for specific selection purposes within the context of fodder oats accessions.

The examination of mean values across various clusters revealed that clusters III and IV displayed superior cluster means for most of the traits. Therefore, clusters III and IV are viable candidates for selecting genotypes to serve as promising parents for hybridization. Notably, genotypes OL-9 (T9), JHO-822 (T22), OL-1937 (T10), OL-1963 (T11), and OL-125 (T5), which belong to these clusters, present an opportunity for inclusion in a crossbreeding program. This strategic selection can potentially yield a high heterotic response, consequently resulting in improved segregants in subsequent generations, particularly with regard to dry matter yield in forage oat.

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Table 1 List of oat genotypes used in the study.

Treatment	Accession name	Species	Treatment	Accession name	Species
1.	OL-1977	*Avena sativa* L.	16.	OL-10	*Avena sativa* L.
2.	OL-1874-1	*Avena sativa* L.	17.	OL-1974	*Avena sativa* L.
3.	OL-1942	*Avena sativa* L.	18.	OL-1967	*Avena sativa* L.
4.	OL-1976-1	*Avena sativa* L.	19.	OL-2000	*Avena sativa* L.
5.	OL-125	*Avena sativa* L.	20.	OL-13	*Avena sativa* L.
6.	OL-1944	*Avena sativa* L.	21.	OL-1896	*Avena sativa* L.
7.	OL-11	*Avena sativa* L.	22.	JHO-822	*Avena sativa* L.
8.	OL-1952	*Avena sativa* L.	23.	AVT-1	*Avena sativa* L.
9.	OL-9	*Avena sativa* L.	24.	OL-212	*Avena sativa* L.
10.	OL-1937	*Avena sativa* L.	25.	OL-1988	*Avena sativa* L.
11.	OL-1963	*Avena sativa* L.	26.	OL-1949	*Avena sativa* L.
12.	OL-1975-2	*Avena sativa* L.	27.	OL-1964	*Avena sativa* L.
13.	OL-15	*Avena sativa* L.	28.	OL-12	*Avena sativa* L.
14.	OL-1802	*Avena sativa* L.	29.	OL-1980	*Avena sativa* L.
15.	OL-1931-1	*Avena sativa* L.	30.	OL-1969	*Avena sativa* L.

Table. 2. Clustering pattern analysis of 50 lines of oat-based on D^2 statistics

Clusters	Genotypes	Number of genotypes
Cluster I	OL-1942 (T2), OL-1944 (T6), OL-1980 (T29), OL-15 (T13), OL-212 (T24), AVT-1 (T23), OL-1952 (T8), OL-1874-1 (T2), OL-1975-2 (T12), OL-1976-1 (T4), OL-12 (T28), OL-1967 (T18), OL-11 (T7), OL-13 (T20)	14
Cluster II	OL-10 (T16), OL-1967 (T18), OL-1977 (T1), OL-1964 (T27), OL-1988 (T25), OL-1896 (T21), OL-1802 (T14), OL- 1974 (17)	8
Cluster III	OL-9 (T9), JHO-822 (T22)	2
Cluster IV	OL-1937 (T10), OL-1963 (T11), OL-125 (T5)	3
Cluster V	OL-1931-1 (T15)	1
Cluster VI	OL-1969 (T30)	1
Cluster VII	OL-1949 (26)	1

Table 3. Intra and Intercluster distances of 30 oat genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	30.71						
Cluster II	58.14	35.71					
Cluster III	57.85	48.82	19.82				
Cluster IV	46.33	61.64	73.91	**42.81**			
Cluster V	69.29	45.44	54.37	83.26	0.00		
Cluster VI	47.99	57.68	77.57	53.78	63.84	0.00	
Cluster VII	100.54	60.83	68.79	**102.31**	64.04	97.52	0.00

Table 4. Cluster means of different yield attributing traits in 30 genotypes of oat

Clusters	DFF	50DF	NTP	NLP	GFY	DMY	LDW	SDW	PH
I	71.09	80.29	8.44	16.67	7.67	1.93	6.61	17.01	128.76
II	71.54	81.24	7.29	14.76	6.65	1.78	6.2	12.39	**117.2**
III	74.37	84.78	8.67	16.69	7.43	1.97	6.96	12.3	135.33
IV	73.35	81.73	9.59	18.52	8.36	2.34	7.51	18.29	152.19
V	73.01	77.95	9.8	15.52	6.4	1.73	4.24	9.5	109
VI	65.11	78.65	8.17	21.03	7.72	2	7.26	17.7	109.03
VII	68.11	80.87	7.31	16.5	5.76	1.75	6.19	8.18	**111.1**
Mean	**70.94**	**80.78**	**8.46**	**17.09**	**7.14**	**1.92**	**6.42**	**13.62**	**123.23**

Clusters	DTM	LSR	CP	CF	TPC	CTC	TAC	PC
I	118.02	0.39	13.96	20.73	181.12	0.72	52.58	1.58
II	121.01	0.5	14.54	22.79	158.42	0.89	36.09	1.17
III	124.26	0.56	13.15	25.99	204.12	0.31	60.6	1.46
IV	121.43	0.41	12.69	21.83	143.54	0.81	27.63	1.72
V	119.09	0.44	16.16	18.48	201.4	1.27	50.98	1.34
VI	119.8	0.41	15.71	20.44	203.13	1.83	40.3	1.63
VII	111.1	0.75	13.65	18.91	127.92	1	41.71	0.85
Mean	**119.24**	**0.49**	**14.26**	**21.31**	**174.23**	**0.97**	**44.27**	**1.39**

DFF: days to first flowering, 50DF: days to 50% flowering, NTP: number of tillers per plant, NLP: number of leaves per plant, GFY: green fodder yield per plot(kg), DMY: dry matter yield per plot(kg), LDW: leaf dry weight per plant (g), SDW: stem dry weight per plant(g), PH: plant height(cm), DTM: days to maturity, LSR: leaf stem ratio, CP: crude protein content, CF: crude fibre content, TPC: total phenolic content, CTC: condensed tannin content, TAC: total antioxidant content, PC: phytate content.

Figure 1. Mahalanobis Euclidean distance in oat genotypes

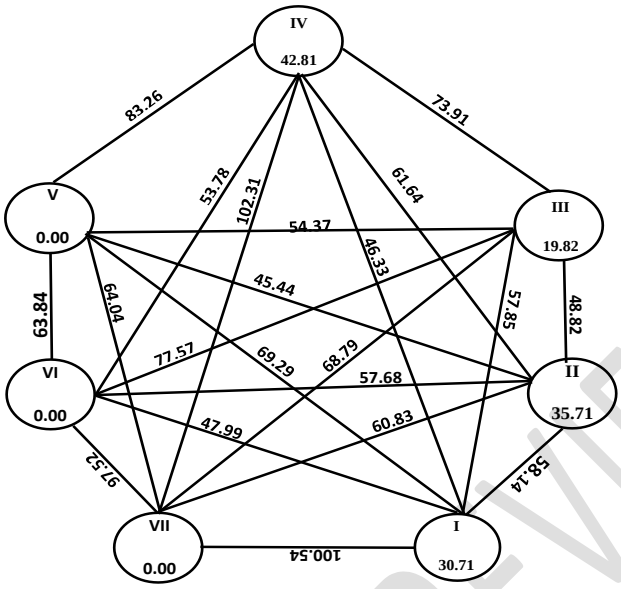

Figure 2. Dendrogram showing the clusters pattern of thirty genotypes of oats