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**Research Article****Evaluation of genetic diversity and forage quality in Alfalfa (*Medicago sativa* L.) germplasm under arid conditions****M. Shahjahan Bukhari¹, Rashid Minhas¹, Rahmat Ullah¹, Mashal Rehman¹, Mahreen Khalid², Ali Abbas³**¹Agricultural Research Station, Bahawalpur, Punjab, Pakistan.²Regional Agricultural Research Institute, Bahawalpur, Punjab, Pakistan.³National Nanfan Research Institute (Sanya), Chinese Academy of Agricultural Sciences, Sanya 572024, China.**ABSTRACT**

Medicago sativa L. commonly known as alfalfa, is a perennial legume crop of significant importance due to its highly nutritional value, and its ability to fix nitrogen in the soil. This study focuses on evaluating the productivity and quality of various alfalfa germplasms under growing conditions using the RCBD design. Statistical analysis such as Augmented RCBD ANOVA, LSD test, genetic advance percentage, heritability percentage, and correlation analysis were performed in this experiment. Key agronomic and quality parameters were recorded, including germination percentage, crop stand, plant height, number of tillers per plant, number of tillers per meter row, and number of leaves per tiller. The quality traits measured were crude protein percentage, starch percentage, ash percentage, crude fiber percentage, moisture dry matter percentage, fat percentage, NDF percentage, and ADF percentage. In 2022, the seeds of 67 germplasms and one check (Sargodha Alfalfa 2011) were evaluated. In 2023, seeds of 214 germplasms and the same check (Sargodha Alfalfa 2011) were evaluated. During 2022, four germplasm lines Acc.#6477, Acc.# 6333, Acc.#6359, and Acc.#6394 showed better performance in terms of alfalfa germination percentage. In 2023, five germplasm lines Acc.#6349, PI 631584, PI 631563, PI 672793, and PI 672804 performed better. Overall, this research provides valuable insights into the genetic variability and potential breeding strategies for improving alfalfa germplasm. These nine germplasms can be grown in Pakistan to achieve better production and quality.

Keywords: *Medicago sativa* L.; genetic advance; heritability; correlation; NDF; ADF.**Correspondence**

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<https://creativecommons.org/licenses/by/4.0>**INTRODUCTION**

Alfalfa (*Medicago sativa* L.) is among the most popular perennial leguminous crops in the world and is commonly known as the “Queen of Forages”. Its high nutritional value, resistance to various climatic conditions, and the ability to fix atmospheric nitrogen make it highly valuable (Burezq et al., 2022). Alfalfa is an important component of sustainable agriculture and serves as a primary source of forage for livestock, particularly in the production of high-quality hay, silage, and pasture. Nevertheless, genetic variability, environmental conditions, and management practices greatly affect the productivity and quality of alfalfa (Singer et al., 2018). Breeding programs have introduced several cultivars derived diverse genetic materials worldwide. These cultivars exhibit specific genetic traits that determine production yield, stability, and the nutritional value of the harvested feed (Capstaff et al., 2018). Identifying desirable traits in alfalfa breeding requires consideration of multiple overlapping factors such as adaptability, appropriate winter dormancy, resistance to pests, grazing tolerance, and the ability to withstand poor soil conditions acidity, aluminium toxicity, and drought stress. The effects of climate change pose a serious threat to the cultivation of alfalfa (luceme) in Pakistan, and

negatively impacting the plant growth, yield, and nutritional quality (Latif et al., 2023). The triple threat of climbing temperatures, erratic rainfall patterns, and an increase in the frequency of droughts increases the risk of a decrease in soil's ability to retain water, resulting in reduced agricultural output (Latif et al., 2023). Selection of seed types is further complicated by increased episodes of extreme weather, including heatwaves and sudden frosts, which hinder germination and crop growth (Pandey et al., 2023). Although high concentrations of carbon dioxide can enhance plant growth under certain conditions, they have adverse effects on forage quality due to shift in protein and fiber content (Verma et al., 2022). Changing climatic conditions have also led to an increase in pest occurrence in alfalfa crops (Verma et al., 2022). To promote sustainable agriculture in Pakistan, farmers are encouraged to adopt drought resistant alfalfa varieties, implement efficient irrigation strategies, and utilize climate smart agricultural technologies that help in maintain stable productivity (Qureshi et al., 2021).

The genetic diversity of alfalfa plant, commonly known as alfalfa, serves as a foundation for its adaptability, resilience, and high production potential (Talla et al., 2023). The autotetraploid nature and high heterozygosity of alfalfa enable researchers to access the extensive genetic variation required for breeding programs aimed at yield enhancement, drought and disease resistance, and improved forage quality. The genetic diversity of alfalfa depends on three major factors: its place of origin, breeding methods, and natural environment. Ancestral wild varieties and traditional local types also serve as fundamental genetic resources (Ghaleb et al., 2021). The assessment and enrichment of alfalfa diversity are made possible through the increasing use of molecular markers, genomic selection, and biotechnological tools. The preservation and utilization of genetic diversity in alfalfa contribute to the development of improved varieties that can withstand environmental changes, soil salinization, and pest challenges, thereby supporting sustainable forage cultivation worldwide. The agricultural characteristics, along with quality aspects, determine the productivity, adaptability, and nutritional value of alfalfa (Tlahig et al., 2024). Crop growth, survival, and forage production under different environmental conditions depends on agronomic traits, including germination rate, plant height, number of tillers per plant, biomass yield, drought tolerance, and pest resistance. The productivity and sustainability of alfalfa rely on these traits for successful establishment, persistence, and yield stability. However, its value as a high-quality forage depends on quality traits such as crude protein content, fiber composition (including NDF and ADF), ash content, and digestibility.

(Baxevanos et al., 2024). A combination of high protein content and balanced fiber composition in alfalfa provides nutritional benefits that enhance animal digestion, thereby boosting milk and meat production. Effective forage production through alfalfa cultivation depends on achieving an appropriate balance between agronomic and quality traits, as output must deliver both productivity and nutritional benefits (Tucker et al., 2024). The breeding process focuses on developing alfalfa varieties that achieve high yields, possess valuable nutritional content, and offer resistance to environmental stresses.

Although of great significance, scant research has been conducted on the breeding practices of alfalfa in various agro-ecological zones of Pakistan. The majority of studies available are general agronomic, yet there is a paucity of specific research to cover the integration of genetic diversity with local climatic stresses like heat, drought and salinity. The gap indicates the necessity of systematic analysis of alfalfa germplasm and breeding strategies that are relevant to the conditions in Pakistan.

Alfalfa production in Pakistan has been limited by drought, salinity, and temperature extremes; however, breeding has not been a top priority compared to staple crops. The use of local germplasm collections is not being exploited, and importation of cultivars has generally led to poor adaptability and failure to deliver reliable yields. It has been clearly demonstrated through literature analysis that there is an urgent need to develop breeding programs that utilize drought- and salinity-resistant varieties of alfalfa, which are compatible with Pakistan's agro-climatic conditions (Qureshi et al., 2021; Latif et al., 2023). However, an acute gap exists in the literature regarding systematic research on these issues; therefore, this study aims to address this gap.

MATERIALS AND METHODS

Experimental Area

The experiment was conducted in 2022-2023 at the Agricultural Research Station, Bahawalpur, Pakistan. A Randomized Complete Block Design (RCBD) was used for this experiment. During 2022, 67 accessions of Alfalfa germplasm and one check variety (Sargodha alfalfa 2011) and During 2023, 214 accessions of Alfalfa germplasm and one check variety (Sargodha alfalfa 2011) were sown to check their yield potential. The soil of ARS, Bahawalpur is sandy loam to clay loam, and this region has high temperatures in summer.

Data Recorded

During the 2022 and 2023 growing seasons, various agronomic and quality parameters of alfalfa germplasm were recorded at appropriate times. The agronomic traits measured included Germination %, crop stand, plant height (cm), no. of tillers/plant, no. of tillers/meter row, and leaves/tillers. The quality traits are crude protein (%), starch (%), ash (%), crude fiber (%), moisture dry matter (%), fat (%), NDF %, and ADF %.

Statistical Analysis

The recorded data were analyzed using R studio programming and statistix 8.1 (Jones et al., 2024). Mean results were considered highly significant at a p-value of 0.01, significant at a p-value of 0.05, and non-significant at a p-value greater than 0.05. The augmented design RCBD (Randomized Complete Block Design) was analyzed, including Least Significant Differences (LSD), heritability %, genetic advance % calculations, and correlation analyses (Kiros Simone et al., 2021). The RCBD design was chosen to reduce the environmental variation between the replications, in order to produce valid comparisons between the genotypes. The treatment differences were tested using ANOVA with LSD due to the method being commonly used in plant breeding to identify significant variation. The selected p-value cut-offs (0.05 and 0.01) are statistically significant. The statistical findings with the practical potential of breeding improvement, heritability and genetic advance and correlation analysis were provided.

RESULTS AND DISCUSSION

In the 2022 growing season, we sowed germplasm accessions numbered 1-70 at the Agricultural Research Station, Bahawalpur. Germination %, crop stand, plant height (cm), no. of tillers/plant, no. of tillers/meter row, leaves/tillers, crude protein (%), starch (%), ash (%), crude fiber (%), moisture dry matter (%), fat (%), NDF %, and ADF % were recorded during this year.

In the 2023 growing season, we sowed germplasm accessions numbered 1-217 at the same location.

Table 1. Analysis of variance during 2022.

	Df	Germ ination	Crop stand	Plant Height	No of tillers /plant	No of tillers / meter row	Leaves/ Tillers	Crude Protein	Starch	Ash %	Crude Fiber	Moistur e Dry Matter	Fat %	NDF	ADF
germplasm	69	228.4	7.64	9.66	1.22	331.74	95.68	2.04	6.78	0.14	9.7	0.06	28.12	10.73	3.17
Control	2	60.11	10.36	1.76	0.08	29.2	4.96	1.63	5.69	0.00	0.1	0.02	0.57	0.44	2.51
Augmented	66	206.0	6.78	8.33	1.04*	338.07*	82.73*	1.73	5.75	0.13*	8.15***	0.054	22.85*	8.56*	2.66
Control v s augmented	1	2044.23**	58.93*	113.6**	15.44**	518.92*	1131.94***	23.42*	77.415*	1.22**	133.00**	0.68*	431.33**	174.81***	38.39*
Error	4	81.71	9.27	2.95	0.24	42.25	8.85	2.66	8.69	0.00	0.09	0.04	1.78	1.25	4.07
CV		13.30%	4.40%	3.80%	3.40%	7.90%	2.70%	7.90%	71.95	0.10%	1%	15.80%	4.80%	5.10%	9.40%
Means		67.96	69.59	45.3	14.59	82.38	109.53	20.76	4.09	13.36	30.92	1.32	27.69	22.12	21.36

Germination Percentage

During the 2022 season, table (1) shows significant differences in germplasm. The plant germination average was 67.961% at a low coefficient of variation of 13.30%. The germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 demonstrated the highest germination rates at 99.36%, 86.36%, 86.11% and 85.33%, respectively in Figure 1. The heritability for the germination rate was 47.31%, and the genetic advance value was 21.46% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. Plant germination averaged 66.20% at a low coefficient of variation of 0.08%. The germplasm ACC.# 6349 along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the highest germination rates at 90.5%, 89%, 88.33%,87.5%, and 84.5%, respectively Figure 1. The heritability measurement for the germination rate was 64.63%, and the genetic advance value was 120.56% in table (3).

Crop Stand

During the 2022 season, table 1 shows significant differences in germplasm. Crop stand averaged 67.961 at a low coefficient of variation of 13.30%. The germplasm ACC.# 6477 along with Acc, # 6333, Acc.#6359 and Acc.#6394 demonstrated the highest crop stand rates at 99.36%, 86.36%, 86.11% and 85.33%, respectively Figure (2). The heritability measurement for the crop stand rate was 47.31%, and the genetic advance value was 21.46% in table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The crop stands of germplasm average was 71.07% at a low coefficient of variation of 9.8%. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793,

and PI 672804, demonstrated the highest crop stand rates at 89.6%, 89.6%, 89.6, 89.5, and 86.6%, respectively Figure 2. The heritability measurement for the crop stand rate was 1.64%, and the genetic advance value was 14.11% in table 3. Table 2. Analysis of variance during 2023.

	Df	Germination	Crop stand	Plant Height	No of tillers /plant	No of tillers / meter row	Leaves /Tillers	Crude Protein	Starch	Ash %	Crude Fiber	Moisture Dry Matter	Fat %	NDF	ADF
Germplasm	69	228.47	7.642	9.66	1.22	331.74	95.68	2.04	6.78	0.14	9.72	0.06	28.12	10.73	3.17
Control	2	60.11	10.36	1.764	0.08	29.2	4.96	1.63	5.69	0.00	0.1	0.028	0.57	0.44	2.51
Augmented	66	206.06	6.78	8.33	1.04*	338.07*	82.73*	1.73	5.75	0.13**	8.15**	0.05	22.85*	8.56*	2.66
Control vs augmented	1	2044.23**	58.93*	113.65*	15.44**	518.92*	1131.94***	23.42*	77.41*	1.22**	133.00***	0.68*	431.3***	174.81***	38.39*
error	4	81.71	9.27	2.95	0.246	42.25	8.85	2.66	8.69	0.0	0.09	0.04	1.78	1.25	4.07
CV		13.30%	4.40%	3.80%	3.40%	7.90%	2.70%	7.90%	71.95	0.10%	1%	15.80%	4.80%	5.10%	9.40%
Means		67.96	69.59	45.30	14.59	82.38	109.53	20.76	4.09	13.36	30.92	1.32	27.69	22.12	21.36

Table 3. Standard error, genetic advance, and heritability 2022-2023.

Trait	Standard Error										During 2022 heritability %	During 2022 genetic advance %	During 2023 heritability %	During 2023 genetic advance %
	8.781	9.304	6.532	7.007	7.436	7.848	4.726	6.364	7.653	47.31				
Germination	8.781	9.304	6.532	7.007	7.436	7.848	4.726	6.364	7.653	47.31	21.46	64.63654	120.5661	
Crop stand	6.777	6.859	6.636	7.120	7.055	5.788	6.918	9.617	4.632	44.34	22.97	1.643352	14.11325	
plant Height	4.171	4.453	4.665	5.085	5.614	6.373	2.802	0.990	4.217	59.86	8.33	42.14445	37.75302	
No.of tillers	0.742	0.725	0.430	0.468	0.434	0.443	0.529	0.141	0.434	95.87	8.21	52.4716	25.89466	
No.of tillers	5.640	5.209	5.575	6.028	6.405	5.417	5.968	0.707	3.564	95.91	44.32	52.4716	11.03311	
Leaves/til	11.035	11.530	11.131	11.397	12.240	9.892	10.281	13.718	10.433	12.32	14.23	39.33261	60.84186	
crude prot	1.112	1.140	1.089	1.015	0.921	1.062	1.184	1.619	1.101	27.27	3.75	98.59155	23.6128	
Starch%	1.282	0.300	1.145	1.228	1.270	1.467	1.061	0.389	1.184	13.15	17.33	67.74959	83.1605	
Ash%	0.720	0.766	0.815	0.874	0.959	1.076	1.256	1.534	0.832	6.12	4.56	12.30837	6.67675	
Crude fibre	1.900	1.953	1.956	1.809	2.017	2.021	2.328	2.786	1.973	11.65	7.65	96.92511	33.24123	
Moisture	0.144	0.150	0.161	0.151	0.161	0.163	0.176	0.099	0.150	3.39	1.28	95.55024	30.31756	
Fat%	4.058	4.307	4.114	4.358	4.568	5.272	6.248	5.636	4.220	27.82	10.54	96.17138	77.28424	
NDF%	2.049	2.169	1.806	1.453	1.225	1.277	0.784	0.205	0.205	12.5	3.63	55.31915	51.59717	
ADF%	1.434	1.497	1.437	1.435	1.570	1.806	1.800	0.976	1.327	85.59	14.45	91.32396	31.52974	

Plant Height

During the 2022 season, table 1 shows significant differences in germplasm. average plant height was 45.307 at a low coefficient of variation of 13.30%. The germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 demonstrated the highest plant length at the rate of 51.94%, 49.94%, 49.4% and 48.6%, Figure 3. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the plant height rate was 59.86%, and the genetic advance value was 8.33% in table 3.

During the 2023 season, table 2 shows significant differences in germplasm. The plant height of average germplasm was 46.46 at a low coefficient of variation of 0.049%. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the highest plant height at 58.93%, 58.93, 57%, 56.6, and 55.86% respectively Figure 3. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the plant height was 42.14%, and the genetic advance value was 37.75% in Table 3.

No. of Tillers/Plant

During the 2022 season, Table 1 shows significant differences in germplasm. The average number of tillers per plant was 14.592 at a low coefficient of variation of 3.4%. The germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 demonstrated the more number of tillers per plant at 16.42, 16.12%, 16.39% and 16.12%, respectively Figure 4. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the number of tillers per plant rate was 95.87%, and the genetic advance value was 8.21% in table 3.

During the 2023 season, table 2 shows significant differences in germplasm. The number of tillers per plant of germplasm average was 14.31 at a low coefficient of variation of 0.048%. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the greater number of tillers per plant at 20.1, 19, 19,18.7, and 18 respectively Figure 4. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the number of tillers per plant was 52.47%, and the genetic advance value was 25.89% in Table 3.

No. of Tillers/Meter Row

During the 2022 season, Table 1 shows significant differences in germplasm. The average number of tillers per meter row was 82.38 at a low coefficient of variation of 7.90%. The germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 demonstrated the more number of tillers per meter row at 95.211%, 93.011% and 93.01%, respectively in Figure 5. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the number of tillers per meter row was 95.91%, and the genetic advance value was 44.32% in table 3.

During the 2023 season, table 2 shows significant differences in germplasm. The number of tillers per meter row of germplasm average was 82.13 at a low coefficient of variation of 0.053%. he germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the highest number of tillers per meter at 99.60, 99.60, 99.04, 98.84, and 98.84, respectively Figure 5. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the number of tillers per meter row was 52.471%, and the genetic advance value was 11.033% in table 3.

Leaves Per Tillers

Table 1 shows significant differences in germplasm during the 2022 season. The average leaves per tiller was 109.5, with a low coefficient of variation of 2.70%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated the most leaves per tiller at 99.86, 98.26, 94.86, and 94.26, respectively, in and Figure 6. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the leaves per tillers was 12.32%, and the genetic advance value was 14.23% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The leaves per tiller of germplasm average was 108.41 at a low coefficient of variation of 0.055%. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the highest leaves per tiller at 99.63, 99.63, 99.43, 99.42, and 99.42 respectively in Figure 6. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the leaves per tillers was 39.332%, and the genetic advance value was 60.84% in table 3.

Crude Protein%

Table 1 shows significant differences in germplasm during the 2022 season. The average of crude protein was 20.76%, with a low coefficient of variation of 7.90%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated the more crude protein at 23.59, 23.11, 22.81, and 22.58, respectively, Figure 7 The standard error value of each germplasm was given in Table 3, and the heritability measurement for the crude protein was 27.27%, and the genetic advance value was 3.75% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The crude protein of germplasm average was 19.83% at a low coefficient of variation of 0.005%. he germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the highest crude protein at 24.17, 24.13, 24.13, 24.12, and 23.65, respectively in and Figure 7. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the crude protein was 98.59%, and the genetic advance value was 23.612% in Table 3.

Starch%

Table 1 shows significant differences in germplasm during the 2022 season. The average of starch was 4.099%, with a low coefficient of variation of 71.85%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated the more starch at 8.74%, 8.37%, 7.95%, and 7.87%, respectively Figure 8. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the starch was 13.15%, and the genetic advance value was 17.33% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The starch of germplasm average was 3.14% at a low coefficient of variation of 0.18%. he germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the more starch at 8.89, 8.89, 8.20, 8.20, and 7.99, respectively Figure 8. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the starch was 67.74%, and the genetic advance value was 83.160% in Table 3.

Ash%

Table 1 shows significant differences in germplasm during the 2022 season. The average of ash was 13.36%, with a low

coefficient of variation of 0.10%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated more ash at 13.76%, 13.71%, 13.71%, and 13.71%, respectively Figure 9. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the ash was 6.12%, and the genetic advance value was 4.56% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The ash of germplasm average was 13.25% at a low coefficient of variation of 0.037%. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the more ash at 14.065%, 14.02%, 14.01%, 14.01%, and 13.96%, respectively in and Figure 9. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the ash was 12.30%, and the genetic advance value was 6.67% in Table 3.

Crude Fiber%

Table 1 shows significant differences in germplasm during the 2022 season. The average of crude fiber % was 30.92%, with a low coefficient of variation of 1%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated more ash at 34.84%, 34.69%, 34.58%, and 34.39% Figure 10. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the crude fiber was 11.65%, and the genetic advance value was 7.65% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The crude fiber of germplasm average was 31.04% at a low coefficient of variation of 0.008%. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the cruder fiber at 35.85, 35.85, 35.58, 35.34, and 35.28, respectively Figure 10. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the crude fiber was 96.92%, and the genetic advance value was 33.24% in Table 3.

Moisture Dry Matter%

Table 1 shows significant differences in germplasm during the 2022 season. The average of moisture dry matter was 1.32%, with a low coefficient of variation of 15.80%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated high moisture dry matter at 1.72%, 1.67%, 1.66% and 1.65%, respectively, in and Figure 11. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the moisture dry matter was 3.39%, and the genetic advance value was 1.28% in table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The moisture dry matter of germplasm average was 1.27% at a low coefficient of variation of 0.043%. he germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the more moisture dry matter at 2.92%, 2.46%, 2.46%, 2.46%, and 2.10%, respectively Figure 11. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the moisture dry matter was 95.55%, and the genetic advance value was 30.31% in table 3.

Fat%

Table 1 shows significant differences in germplasm during the 2022 season. The average of fat % was 27.69, with a low coefficient of variation of 4.80%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated more fat at 35.88%, 34.80%, 34.79% and 34.69%, respectively Figure 12. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the fat was 27.82%, and the genetic advance value was 10.54% in table 3.

During the 2023 season, table 2 shows significant differences in germplasm. The fat of germplasm average was 30.99% at a low coefficient of variation of 0.021%. he germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the more fat at 38.94%, 38.6%, 38.6%, 38.4%, and 38.35%, respectively Figure 12. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the fat was 96.17%, and the genetic advance value was 77.28% in Table 3.

NDF %

Table 1 shows significant differences in germplasm during the 2022 season. The average of NDF % was 22.12%, with a low coefficient of variation of 5.10%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated more NDF at 28.19%, 27.11%, 26.61%, and 26.32%, respectively Figure 13. The standard error value of each germplasm was given in Table 3, and the heritability measurement for the NDF was 12.5%, and the genetic advance value was 3.63% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The NDF% of germplasm average was 23.42% at a low coefficient of variation of 0.072. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the more NDF at 31.245%, 30.82%, 30.82%, 30.75%, and 30.56% respectively Figure 13.

The standard error value of each germplasm was given in Table 3, and the heritability measurement for the NDF % was 55.319%, and the genetic advance value was 51.59% in Table 3.

ADF %

Table 1 shows significant differences in germplasm during the 2022 season. The average of ADF % was 21.36%, with a low coefficient of variation of 9.40%. The germplasm ACC.# 6477, along with Acc, # 6333, Acc.#6359, and Acc.#6394, demonstrated more ADF at 25.491%, 24.74%, 24.13%, and 24.11%, respectively Figure 14. The standard error value of each germplasm was given in Table 3, and the standard error, heritability measurement for the ADF was 85.59%, and the genetic advance value was 14.45% in Table 3.

During the 2023 season, Table 2 shows significant differences in germplasm. The average ADF of germplasm was 20.25%, with a low coefficient of variation of 0.016%. The germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804, demonstrated the more ADF at 26.18, 26.18, 25.73, 25.73, and 24.82, respectively Figure 14. The standard error value of each germplasm was given in Table 3, and the standard error, heritability measurement for the ADF% was 91.23%, and the genetic advance value was 31.52% in Table 3.

Correlation 2022

The Pearson correlation matrix shows variable relationships through colored circular representations. The blue circles in the correlation matrix display strong positive relationships between variables which reach values near 1 where ADF and NDF and CF and ADF show such associations while, the red circles represent negative relationships between variables that reach values near -1, and GER and ADF and GER and NDF display these patterns in Figure 15. The strength of a relationship between variables becomes more apparent through larger diameters of the circles, while smaller diameters indicate weaker relations. The matrix shows important associations between variables that require additional investigation.

Correlation 2023

The color coded and sized circular representation of the Pearson correlation matrix shows the variable relationships in the dataset. The blue circle shows positive relationships that arise from PH and NTP data and from NTP and NTM, and also from STR and ASH. One variable's growth contributes to an increase in the other variable, while red circle FAT shows a strongly negative relationship with NDF and ADF, and also has this connection with NDF in Figure 16. The matrix shows that FAT increases while NDF and ADF decrease in opposite directions, and some moderate levels of positive and negative correlation exist between those variable pairs. The matrix allows researchers to detect important variable connections which should be studied further.

The two-year trials from 2022 to 2023 showed significant differences in the germination of the germplasm. The germination rate plays a crucial role in normal alfalfa growth. A higher percentage of germination shows better tolerance, while a lower germination rate shows less resistance. The middle-ranged germination shows good potential to develop alfalfa germplasm that performs better in normal conditions (Nan et al., 2019). Genetic factors influencing alfalfa yields were found to be moderate in 2022 but showed restricted potential for improvement, while on the other hand, 2023 highlighted stronger genetic effects and greater opportunity for alfalfa varieties selection through selective breeding (Aruna et al., 2023; Jade et al., 2023). The two-year trials from 2022 to 2023 showed significant differences in the germplasm of crop stand percentage. The crop stand rate has a crucial role in normal alfalfa growth. A higher percentage of crop stand shows better tolerance, while a lower crop stand rate shows less resistance. The middle-ranged crop stand shows good potential to develop alfalfa germplasm that performs better in normal conditions. The data from 2022 demonstrates that heredity plays a moderate role in this condition alongside available breeding potential. However, the results from 2023 point toward low genetic components and constrained breeding potential for future improvement (Aruna et al., 2023; Jade et al., 2023). The two-year trials from 2022 to 2023 showed significant differences in the germplasm of plant height percentage. The plant height rate has a crucial role in normal alfalfa growth. A higher plant height shows better tolerance, while a lower plant height shows less resistance. The moderate plant height shows good potential to develop alfalfa germplasm that performs better in normal conditions. The study results from 2022 showed heritability was high yet advancements through breeding methods appeared challenging whereas in 2023 the findings implied heritable characteristics remained strong yet breeding could bring more improvement potential (Aruna et al., 2023; Jade et al., 2023). The two-year trials from 2022 to 2023 showed significant differences in the germplasm of the number of tillers per plant. The number of tillers per plant has a crucial role in normal alfalfa growth. More number of tillers per plant shows better tolerance, while a few numbers of tillers per plant shows less resistance. The moderate number of tillers per plant shows good potential to develop alfalfa germplasm that performs better in normal conditions. Genetic control of the trait appeared dominant in 2022 although changed in 2023 to show genetic potential for improvement through breeding programs despite the mode

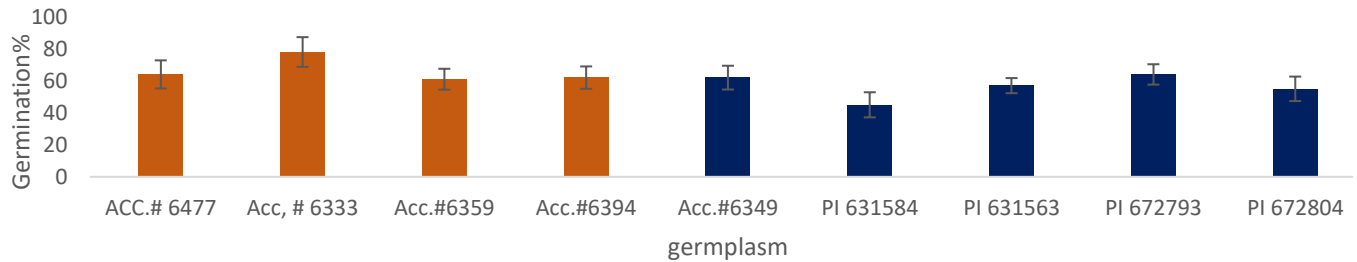


Figure 1. During 2022, sown 70 germplasm, only these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in germination rate, while during 2023, sown 217 germplasm but, these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

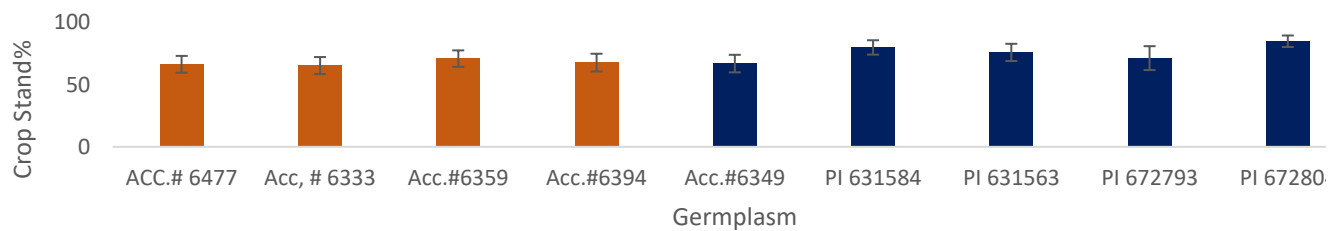


Figure 2. During 2022, sown 70 germplasm, only these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in crop stand, while during 2023, sown 217 germplasm but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

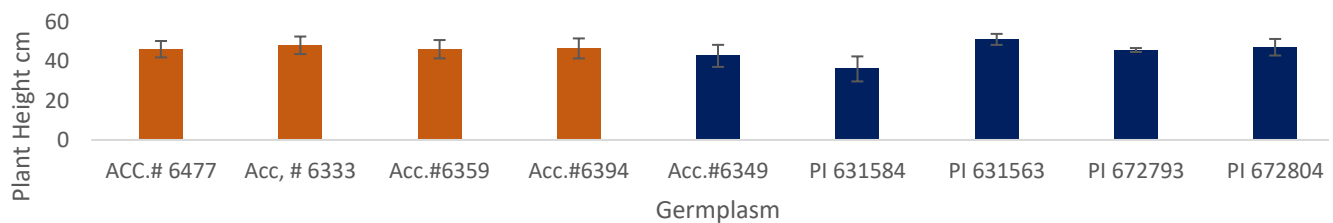


Figure 3. During 2022, sown 70 germplasm but these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in plant height, while during 2023, sown 217 germplasm but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

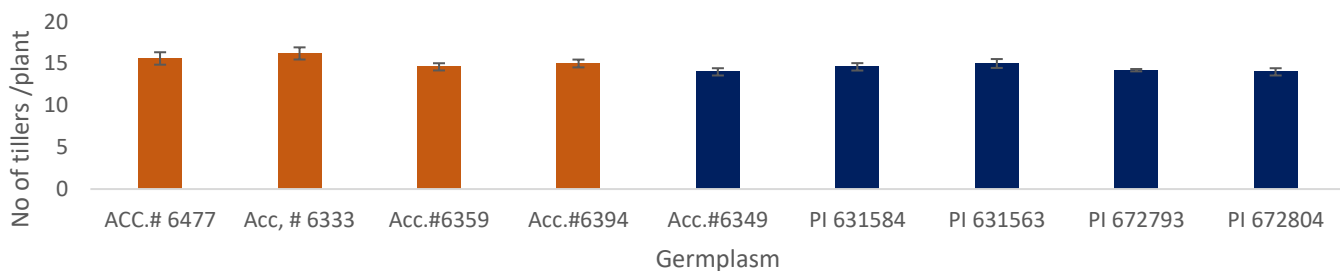


Figure 4. During 2022, sown 70 germplasm, but these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in No. of tillers per plant, while during 2023, sown 217 germplasm, these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal conditions.

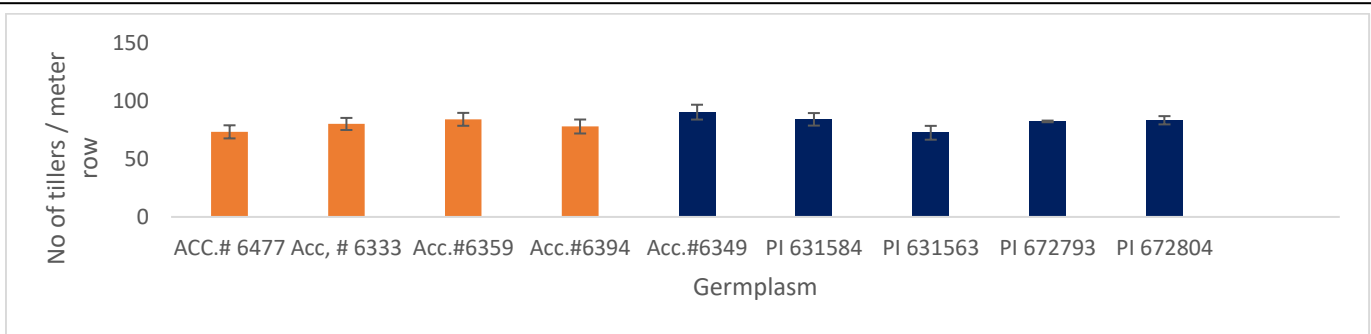


Figure 5. During 2022, sown 70 germplasm but these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in no. of tillers per meters, while during 2023, sown 217 germplasm, but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

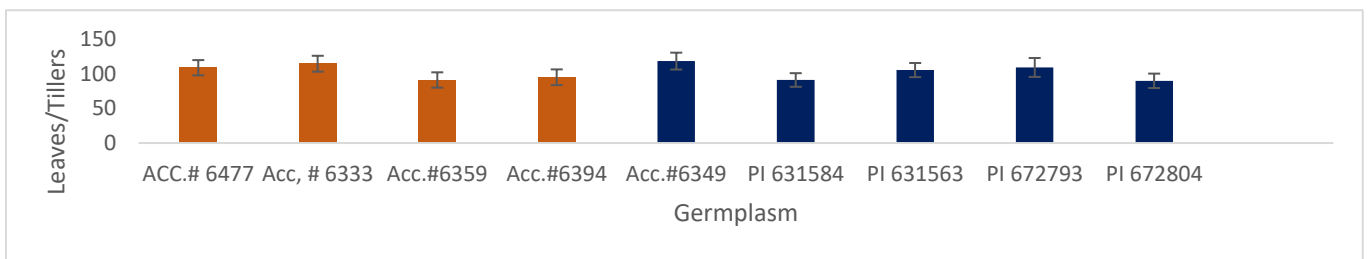


Figure 6. During 2022, sown 70 germplasm, but these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in leaves per tillers, while during 2023 sown 217 germplasm, but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

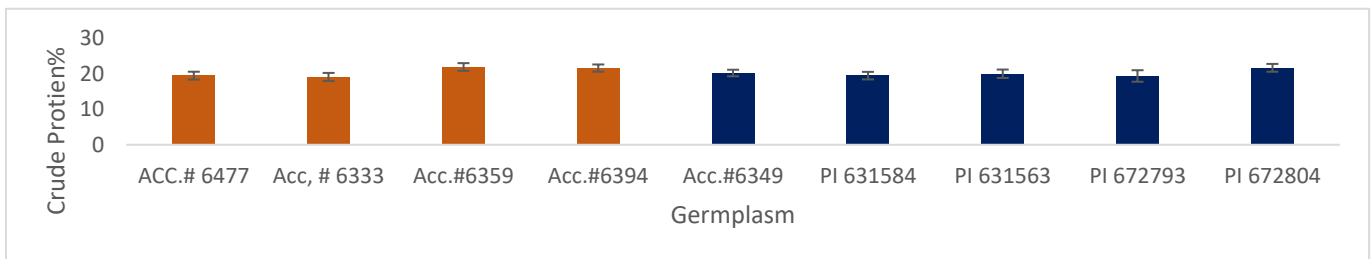


Figure 7. During 2022, sown 70 germplasm, but these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in crude protein%, while during 2023 sown 217 germplasm, but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

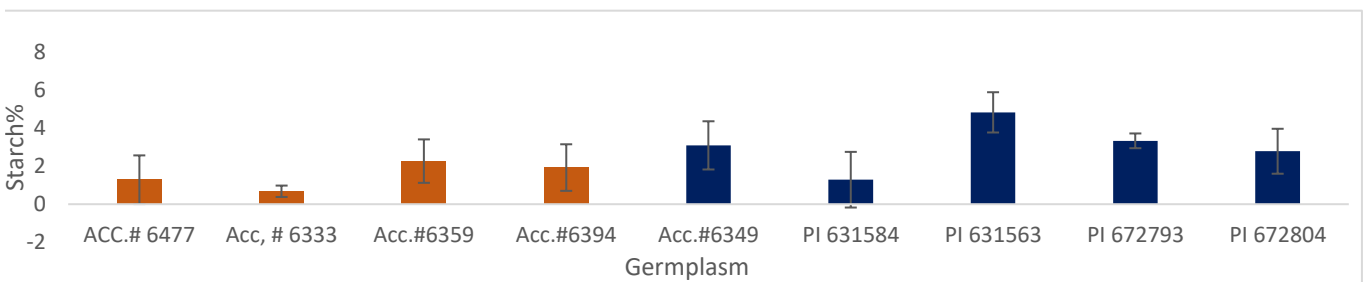


Figure 8. During 2022, sown 70 germplasm but these four germplasm ACC. # 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in starch%, while during 2023, sown 217 germplasm but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

moderate heritability (Aruna et al., 2023; Jade et al., 2023). The two-year trials from 2022 to 2023 showed significant

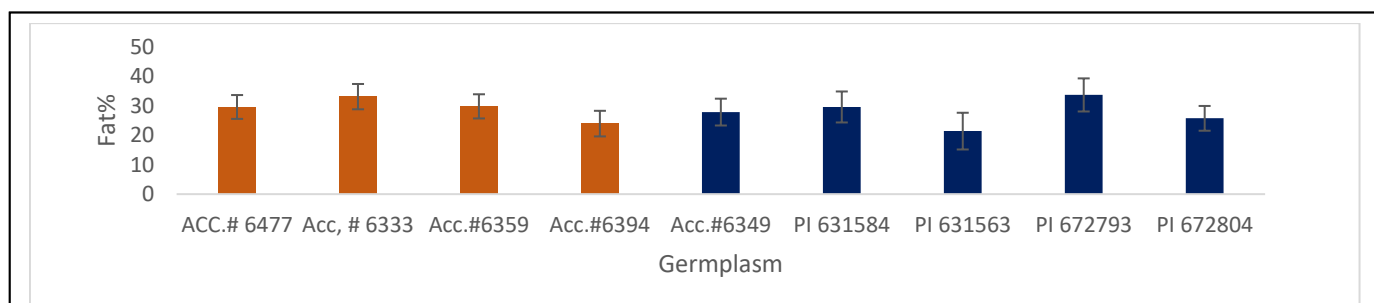


Figure 12. During 2022, sown 70 germplasm these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in fat%, while during 2023, 217 germplasm but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

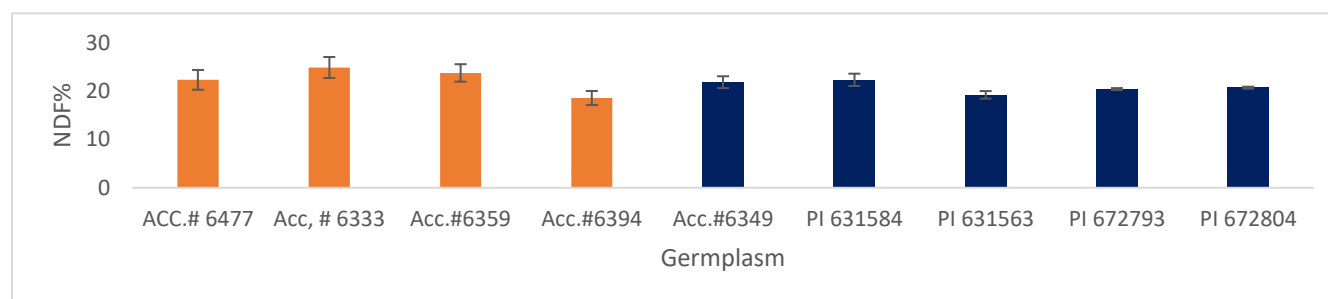


Figure 13. During 2022, sown 70 germplasm but these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in NDF%, while during 2023, sown 217 germplasm but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

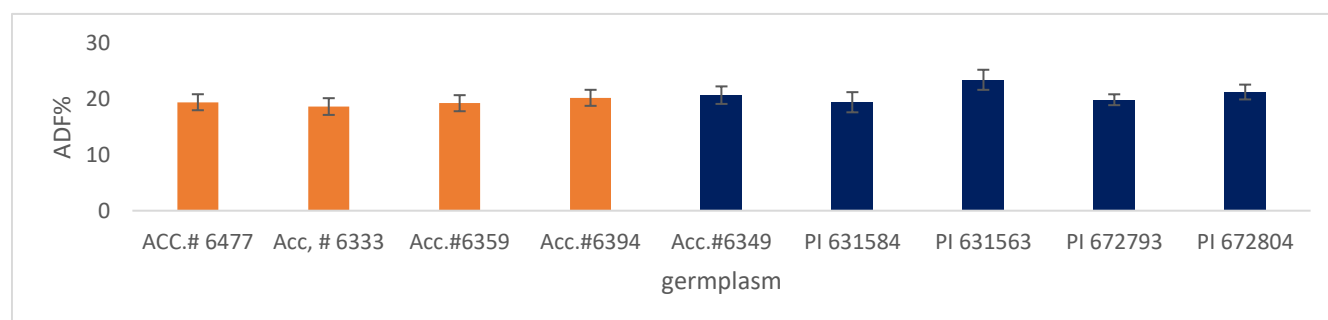


Figure 14. During 2022, sown 70 germplasm but these four germplasm ACC.# 6477 along with Acc, # 6333 and Acc.#6359 and Acc.#6394 perform better in ADF%, while during 2023, sown 217 germplasm but these five germplasm ACC.# 6349, along with PI 631584, PI 631563, PI 672793, and PI 672804 perform better in normal condition.

differences in the germplasm of the number of tillers per meter row. The number of tillers per meter row has a crucial role in normal alfalfa growth. More number of tillers per meter row shows better tolerance, while a few number of tillers per meter row shows less resistance. The moderate number of tillers per meter row shows good potential to develop alfalfa germplasm that performs better in normal conditions. The data from 2022 shows dominant additive genes along with high selection potential for improvement of the trait, but the 2023 results point to environmental factors decreasing breeding efficiency with moderately influenced genetics ((Putnam et al., 2021; Grieder et al., 2021). Two-year trials from 2022 to 2023 showed significant differences in the germplasm of leaves per tiller. The leaves per tiller have a crucial role in normal alfalfa growth. More number of leaves per tillers shows better tolerance, while a few numbers of leaves per tiller show less resistance. The moderate number of leaves per tillers shows good potential to develop alfalfa germplasm that performs better in normal conditions. The data from 2022 indicated environmental factors had complete control over traits but the 2023 data showed additive genetics gained dominance over traits, which made them more suitable for breeding improvements (Aruna et al., 2023; Tlahig et al., 2024). **The** fiber related characteristics (ADF and NDF) have greater heritable control than protein content, supporting the possibility of breeding selectively to enhance forage digestibility (C.

Yang et al., 2021). On the other hand, crude protein and leaf per tiller were traits that exhibited environmental dominance in 2022, and the environmental variability is one of the challenges encountered when trying to enhance nutritional quality. It should be noted that the tendency toward a greater degree of genetic control in 2023 underscores the probability of breeding, according to a particular choice (Jade et al., 2023).

In practical breeding, high heritability breeding characteristics such as plant height, crude fiber, and ADF are best utilized

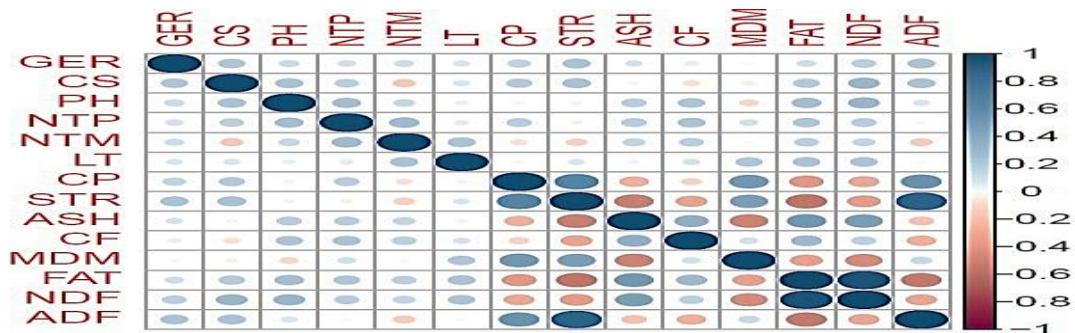


Figure 15. The blue circles in the correlation matrix display strong positive relationships between variables which reach values near 1 where ADF and NDF and CF and ADF show such associations while, the red circles represent negative relationships between variables that reach values near -1, and GER and ADF and GER and NDF

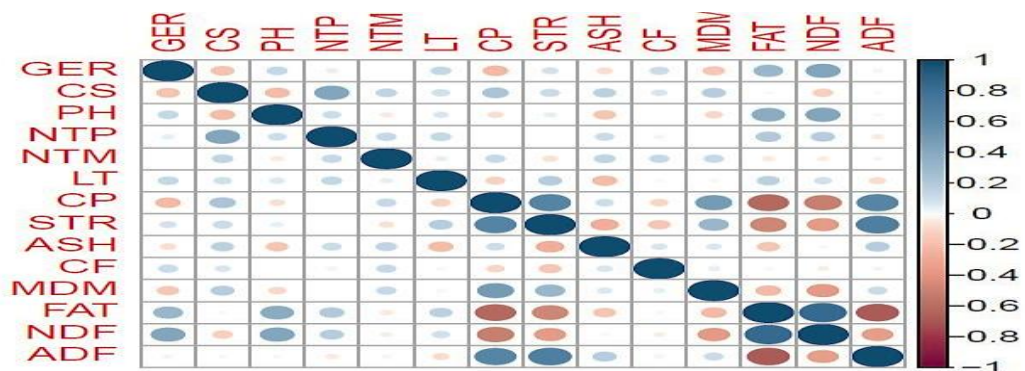


Figure 16. The blue circle shows positive relationships that arise from PH and NTP data and from NTP and NTM and also from STR and ASH. One variable's growth contributes to an increase in the other variable, while the red circle FAT shows a strongly negative relationship with NDF and ADF, and also has a connection with NDF

in early generation selection, with lower heritability breeding characteristics in need of multi-environment testing or genomic-assisted breeding. The correlations between fiber components and nutritional characteristics observed indicate that indirect selection should be used to enhance feed efficiency at a lower cost of measurement.

The two-year trials from 2022 to 2023 showed significant differences in the germplasm of crude protein. The crude protein has a crucial role in normal alfalfa growth. High crude protein shows better tolerance, while less crude protein shows less resistance. The normal crude protein shows good potential to develop alfalfa germplasm that performs better in normal conditions. The same traits tested between 2022 and 2023 showed environmental dominance in 2022 but complete genetic control in 2023, which enabled better trait enhancement outcomes despite modest predicted benefits (Lamb et al., 2014; Fan et al., 2023).

The two-year trials from 2022 to 2023 showed significant differences in the germplasm of starch percentage. The starch has a crucial role in normal alfalfa growth. High starch shows better tolerance, while less starch shows less resistance. The normal starch shows good potential to develop alfalfa germplasm that performs better in normal conditions. The results of 2022 demonstrated environmental factors dominated the traits variation and showed minimal breeding prospects yet 2023 found genetic control was strong with excellent possibilities for selective breeding to improve traits (Aruna et al., 2023; Jade et al., 2023).

The two-year trials from 2022 to 2023 showed significant differences in the germplasm of ash percentage. The ash has a crucial role in normal alfalfa growth. High ash content shows better tolerance, while less ash shows less resistance. The normal ash content shows good potential to develop alfalfa germplasm that performs better in normal conditions. The

genetic factors showed high dominance in 2022, which indicated weak heritability but minimal breed ability, whereas the analysis from 2023 revealed some heritable elements with average potential for genetic improvement (Aruna et al., 2023; Javaid et al., 2020). The two-year trials from 2022 to 2023 showed significant differences in the germplasm of crude fiber percentage. The crude fiber has a crucial role in normal alfalfa growth. High crude fiber content shows better tolerance, while less crude fiber shows less resistance. The normal crude fiber content shows good potential to develop alfalfa germplasm that performs better in normal conditions. The 2022 results indicated environmental dominance led to breeding constraints, yet the 2023 results revealed a significant genetic influence which would facilitate effective selection (Aruna et al., 2023; Jade et al., 2023). The two-year trials from 2022 to 2023 showed significant differences in the germplasm of moisture dry matter percentage. The moisture-dry matter has a crucial role in normal alfalfa growth. High moisture dry matter shows better tolerance, while less moisture dry matter shows less resistance. The normal moisture dry matter shows good potential to develop alfalfa germplasm that performs better in normal conditions. The 2022 result showed strong environmental factors as the main influence, with limited breeding potential. On the other hand, research in 2023 showed strong genetic factors which would support better breeding outcomes through selection (Aruna et al., 2023; Blumenthal et al., 2020).

The two-year trials from 2022 to 2023 showed significant differences in the germplasm of fat percentage. The fat has a crucial role in normal alfalfa growth. High fat show better tolerance, while a lower fat show less resistance. The normal fat shows good potential to develop alfalfa germplasm that performs better in normal conditions. The study during 2022 showed both genetic and limited breeding potential but 2023 research demonstrated complete genetic control for highly effective trait selection through breeding strategies (Aruna et al., 2023; Jade et al., 2023).

The two-year trials from 2022 to 2023 showed significant differences in the germplasm of NDF percentage. The NDF has a crucial role in normal alfalfa growth. High NDF shows better tolerance, while a lower NDF shows less resistance. The normal NDF shows good potential to develop alfalfa germplasm that performs better in normal conditions. The 2022 alfalfa data points toward environmental dominance over breeding potential until 2023 and shows genetic control and substantial breeding benefits through selection (Atumo et al., 2021; Xu et al., 2023).

The two-year trial data from 2022 to 2023 showed significant differences in the germplasm of ADF. The ADF has a crucial role in normal alfalfa growth. High ADF show better tolerance, while a lower ADF show less resistance. The normal ADF show good potential to develop alfalfa germplasm that performs better in normal conditions. The year 2022 research discovered that genes strongly control the traits with minimal potential for enhancement, followed by the year 2023 study, which revealed almost complete genetic control leading to notable improvement through breeding selection (Aruna et al., 2023; Jade et al., 2023).

The nutritional relationships between variables are presented through the correlation matrix in 2022. The analysis of feed materials shows positive connections between fiber components ADF and NDF and between CF and ADF because these components share roles in examination procedures. The relationship between GER shows negative values toward ADF along with NDF because greater fiber content leads to decreased digested energy. The research recommends additional exploration regarding energy prediction models and biological interpretations of fiber interactions with energy resources. During 2023, the present variable relationships are shown through color coded circles in its correlation matrix. The correlation between PH and NTP and between NTP and NTM and between STR and ASH is represented as positive correlations through blue circles, which direct influence between variables. The relationship between FAT and both NDF and ADF shows negative trends, which are demonstrated through red circles. The graphic display helps to locate essential connections which require more examination.

CONCLUSION

Alfalfa (*Medicago sativa* L.) is an important forage crop due to its high nutritional value and ability to add value to soil by boosting the level of nitrogen in the soil through biological nitrogen fixation. This study evaluated diverse alfalfa germplasm over two growing seasons, revealing significant variability in agronomic and quality traits. Plant height, percentage of germination, and nutritional composition were found to be key parameters that may significantly determine the performance. Some of the accessions, including #6477, #6333, #6359, #6394, #6349, PI 631584, PI 631563 and PI 672793 and PI 672804, were observed to perform better than others. These findings provide usable insights for breeding programs aimed at improving forage yield and nutritional value under local conditions. Use of these high yielding germplasm lines has the potential to improve forage production, livestock diets and sustainable agricultural production.

AUTHOR'S CONTRIBUTION

M. ShahJhan made a substantial contribution to the study design, analysis, and acquisition of data. He was also responsible for manuscript writing and gave the final approval of the version to be published. Rashid Minhas gave the final approval of the version to be published. Rahmat Ullah made a substantial contribution to the study design. Mashal Rehman contributed significantly to the study design, acquisition, and interpretation of data, and was actively involved in critical review and manuscript writing, in addition to providing final approval of the version to be published. Mehreen Khalid contributed to the statistical data analysis. Finally, Ali Abbas contributed to data collection.

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AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not required for this study, as it involved only crop research and did not include human or animal subjects.

CONSENT FOR PUBLICATION

Not applicable. This study does not involve any individual person's data in any form. All authors agree to its publication.

CONFLICT OF INTERESTS

All authors attest to the validity of manuscript contents and agree for submission.

ACKNOWLEDGEMENT

Authors have no conflict of Interest regarding this publication.

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