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## DEVELOPMENT OF MULTI-CUT ALFALFA (*MEDICAGO SATIVA* L.) CULTIVARS FOR SALINE SOILS AND HOT CLIMATES UNDER IRRIGATED CONDITIONS

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### SUMMARY

The study comprised a comparative evaluation of 29 accessions of alfalfa (*Medicago sativa* L.) in the control nursery and 21 accessions in the competitive nursery versus the standard cultivar Damu-12 during cropping seasons of 2023–2025 in Turkestan Region, Kazakhstan. Among the 29 accessions tested in the control nursery, 11 accessions (M-2515, M-2520, M-2521, M-2532, M-2579, M-2584, M-2587, M-2588, M-2562, M-2572, and M-2589) significantly exceeded the standard cultivar Damu-12 in green and dry matter yields. These accessions excelled the standard genotype by 110.2%–115.5%, achieving dry hay and green mass yields of 22.7–23.8 t/ha and 127.1–131.9 t/ha, respectively. In the competitive nursery with 21 accessions, 11 accessions (M-2572, M-2584, M-2532, M-2566, M-2537, M-2522, M-2579, M-2552, M-2582, M-2881, and M-2545) revealed the highest green and dry mass yields. Their green and dry mass yields outperformed the standard cultivar Damu-12 by 110.2%–122.9%. Cluster analysis categorized the accessions into three distinct groups: Cluster 1 (high-yield intensive type), Cluster 2 (high forage quality and dry matter), and Cluster 3 (stable/low performance). Clusters 1 and 2 were distinct as primary genetic resources for developing transgressive hybrids that combine rapid biomass accumulation with enhanced nutritional values.

**Keywords:** Alfalfa (*M. sativa* L.), breeding nursery, competitive varietal testing, stem height, leafiness, green mass yield, dry matter yield

**Key findings:** The relevant study identified the high-yielding irrigated alfalfa (*M. sativa* L.) genotypes through control and competitive nurseries. These promising accessions can be useful in future breeding programs for developing high-yielding cultivars for the Turkestan Region, Kazakhstan.

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## INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the leading forage legume cultivated on over 35 million hectares globally. It also surpasses other legumes in nutrient density, producing 1.5–2.0 t of protein per hectare, which is significantly higher than soybean and wheat. Alfalfa remains a globally dominant forage crop due to its superior nutritional profile. At the flowering stage, 100 kg of fresh herbage contains 21–23 feed units and 4.05–4.12 kg of digestible protein, yielding 6.1–6.3 times more protein than soybeans (Kosolapov, 2014).

In arid regions, salt stress poses a major challenge by hindering seed germination and dry matter accumulation. Alfalfa adapts through osmolyte accumulation (proline and soluble sugars), activation of antioxidants, and by maintaining a low ratio of  $\text{Na}^+:\text{K}^+$ . Breeding salt-tolerant genotypes comprises intensive screening for physiological indices and also utilizing functional genomics to identify specific QTLs. Additionally, the use of growth-promoting bacteria, mycorrhizal fungi, and seed priming with various growth regulators like gibberellic acid can considerably boost the alfalfa performance under saline environments (Al-Farsi *et al.*, 2020).

Alfalfa production heavily relies on irrigation practices, approximately 51% in the USA (USDA-NASS, 2018) and almost 100% due to arid conditions in the Turkestan Region (Bureau of National Statistics, 2023). Climate change and expanding irrigation in arid and semi-arid areas exacerbate global salinity issues, with over 30% of alfalfa-growing areas in the USA experiencing severe drought over the last decade (USDA-ERS, 2022). Although some forage grasses, such as *Thinopyrum ponticum*, are more salt-tolerant, they cannot match alfalfa's nutritional value and profitability.

The assessment of irrigation water quality and its potential impact on soil salinity proceeded following the standard FAO (Food and Agriculture Organization) guidelines (Ayers and Westcot, 1985) and established drainage management protocols (Hanson *et al.*, 2006). Management of alfalfa (*Medicago sativa* L.) in arid regions requires specific irrigation

strategies to mitigate the effects of high salinity on forage productivity (Hanson *et al.*, 2007; Benes *et al.*, 2018). The efficiency of the breeding process in selecting promising alfalfa accessions entailed evaluation considering the methodological frameworks for crop improvement (Ceccarelli, 2015).

Soil salinity impairs plant root growth, water infiltration, and oxygen levels (Grattan and Oster, 2003). In irrigation water, the increased salt concentration causes foliar damage and internal physiological injuries in crop plants (Munns and Tester, 2008). Alfalfa's response to salt stress includes a considerable reduction in green and dry biomass, shoot height, and leaf area, with enhanced leaf-to-stem ratio (Sandhu *et al.*, 2017).

At the molecular and biochemical levels, plants respond by upregulating tolerance genes, accumulating soluble sugars, and sequestering toxic  $\text{Na}^+$  and  $\text{Cl}^-$  ions into vacuoles (Munns and Tester, 2008; Mouradi *et al.*, 2018). Furthermore, salinity negatively affects symbiotic nitrogen fixation, reducing nodule count and atmospheric  $\text{N}_2$  fixation; however, these effects can be partially mitigated by low-level nitrogen application (Elgarably and Benes, 2021; Latrach *et al.*, 2014).

Soil salinity is a critical abiotic stress affecting over 100 countries worldwide. According to the United Nations Educational, Scientific and Cultural Organization (UNESCO) and FAO, saline areas have expanded to 932.2 million hectares globally (Rengasamy, 2006). This soil degradation reduces arable lands and pasture quality, intensifying competition for resources between food production and livestock. Consequently, developing salt-tolerant crop cultivars is essential to improve land-use efficiency in saline-alkaline environments (Zhang *et al.*, 2008).

Direct selection under saline field conditions often reveals inconsistent results due to temporal and spatial fluctuations in soil salinity (Flowers and Flowers, 2005). Therefore, effective breeding requires standardized procedures to minimize the uncontrolled variability. A successful approach developed by Peel *et al.* (2004) involves screening plants in a silica sand medium under

increasing salinity levels. Lines developed through this controlled greenhouse method exhibited key adaptive parameters, such as intensive root growth, reduced stomatal conductance, maintenance of relative water content via soluble sugar accumulation, and effective ionic homeostasis with lower Na<sup>+</sup> accumulation (Anower *et al.*, 2013, 2017).

Crop breeding mainly aims to minimize the genotype-by-environment interactions (GEI) in studying different crops under diverse environments (Simmonds, 1991). However, in salt-stressed environments, the decline in productivity is nearly inevitable. A practical approach was to identify lines that perform consistently across the stressed and non-stressed environmental conditions, such as CS 7-3 and SII 8-2, which outperformed their parental means in both tests. Conversely, under severe stress conditions, lines BC79S and 5-1 were more beneficial by maintaining higher yields in stress environments despite lower productivity in optimal conditions. The following study aimed to evaluate the 29 accessions of alfalfa (*M. sativa* L.) in the control nursery and 21 accessions in the competitive nursery in comparison with the standard cultivar Damu-12 during the crop seasons of 2023–2025 in the Turkestan Region, Kazakhstan.

## MATERIALS AND METHODS

### Breeding materials and procedure

The alfalfa (*M. sativa* L.) experimental studies began during the crop season of 2023 and continued until 2025 at the Agricultural Experimental Station of Cotton and Melon Growing, Turkestan Region, Kazakhstan. Overall, the breeding material comprised 29 accessions in the control nursery and 21 accessions in the competitive nursery (totaling 50 unique genotypes).

The establishment of experiments followed the Vavilov All-Russian Institute of Plant Genetic Resources (VIR) methodology. The alfalfa cultivar Damu-12 served as the standard (check), sown after every 10 accessions. Damu-12 is a high-yielding, mid-

season cultivar developed for irrigated conditions in Southern Kazakhstan, characterized by good salt tolerance and stable regrowth capacity across multiple cuttings. The preceding crops were cotton and melon. The trial employed a randomized small-plot design with three replications; the total subplot area was 12 m<sup>2</sup> (harvest area of 10 m<sup>2</sup>). Alfalfa accessions sown had a rate of 2 g/m<sup>2</sup>, followed by standard tillage and pest and weed control measures.

Alfalfa productivity and forage quality evaluation focused on plant height, leafiness, green mass, and hay yield. Nutritional values (dry matter, protein, ash, fat, and fiber content) also received analysis. Plant height measurement was at 10 points per plot before harvesting. Green mass harvesting commenced at the budding-to-early flowering stage. Leafiness and qualitative indicators succeeded in their determination from 1-kg representative samples collected during harvest.

### Soil and climate conditions

The experimental site featured light serozem soil with a medium-loamy texture. Soil salinity analysis revealed a total soluble salt content ranging from 0.45% to 0.68%, with a predominance of sulfate-chloride ions, characterizing the site as moderately saline. The electrical conductivity (EC<sub>e</sub>) of the soil extract varied between 4.2 and 8.5 dS/m. Soil analysis (0–20 cm) showed an average humus content of 0.82%–0.85%, mobile phosphorus (12.0–22.0 mg/kg), and potassium (145.0–250.0 mg/kg). The groundwater table (GWT) fluctuated seasonally, rising to 106.6 cm in April and receding to 275.0 cm by September due to the summer-autumn dry period. In the Maktaaral District, the groundwater salinity during 2023–2025 was high, with electrical conductivity (EC<sub>gw</sub>) ranging from 6.4 to 8.2 dS/m (mineralization of 4.1–5.3 g/L). The dominant salts were of the sulfate-chloride type, which created severe osmotic and toxic stress for the alfalfa accessions through capillary rise.

The 2023–2025 period featured extreme continental conditions with summer

temperatures and heatwave frequencies significantly exceeding long-term regional norms. These conditions, which surpassed historical records, provided a rigorous test for the adaptability of the alfalfa accessions. Summer daytime temperatures reached +40 °C to +45 °C, providing extreme hydrothermal conditions to test the adaptability of the alfalfa accessions. Despite these stress conditions, the regional agricultural sector showed a 17.3% increase in gross output by 2025, underlining the practical relevance of developing heat-resistant cultivars. The conduct of this research followed the VIR unified classifier for *Medicago* L. and state variety testing protocols (2015). Statistical analysis used Microsoft Excel. Statistical processing of the experimental data underwent the analysis of variance (ANOVA) according to the methodology of field experimentation (Dospekhov, 1985).

## RESULTS AND DISCUSSION

### Control nursery

In the control nursery, 29 promising accessions of alfalfa (*M. sativa* L.) underwent evaluation against the check cultivar Damu-12 based on cumulative data over five cuttings per crop season (2023–2025). The study confirmed high winter hardiness exceeding 85%, supported by weather data showing absolute minimum temperatures reaching –25 °C to –28 °C with inconsistent snow cover during wintering periods. These results are consistent with the findings of Roumiantseva *et al.* (2022), who emphasized that winter hardiness remains a critical adaptive marker for alfalfa germplasm in the sharply continental climate of Kazakhstan. Morphological analysis showed 23 accessions were upright and six were semi-upright. Tillers' density was a distinguishing factor; 21 genotypes exhibited the highest tiller density (67–70 stems/plant), while eight accessions showed better density (52–55 stems/plant).

The regrowth intervals (inter-cutting periods) were highly responsive to hydrothermal conditions, totaling five harvests per season. The duration from spring onset to

the first cutting was 45 days, followed by subsequent intervals of 36, 33, 38, and 35 days, respectively. A strong synchronization was evident between the regrowth duration and plant height development. For the first cutting (45 days), the elite accessions reached a maximum average height of 88–92 cm. During the subsequent intensive summer cycles (33–36 days), despite shorter intervals, the growth rate accelerated to 2.4–2.7 cm/day, allowing genotypes like M-2561 and M-2582 to maintain a high pre-harvest height (above 85 cm). This demonstrates the high regenerative capacity and intensive growth potential of the studied germplasm under thermal resources of the Turkestan Region. According to Tlahig *et al.* (2021), such responsive regrowth patterns are typical for alfalfa genotypes well-adapted to arid environments outside oases, where hydrothermal resources limit the vegetation period. The results revealed the highest regeneration rate, essential for maintaining stable forage productivity under the arid climate of Southern Kazakhstan.

For agrobiological indicators in the control nursery during 2025, performing a detailed productivity analysis of the 29 promising alfalfa accessions compared with the check cultivar Damu-12 ensued. The analysis (2023–2025) revealed significant variability among alfalfa genotypes in productivity and forage quality parameters (Table 1). Regarding green mass yield, the check cultivar Damu-12 (128.4 t/ha) maintained a leading position; however, the accession M-2520 showed a high competitive potential with 120.4 t/ha, demonstrating better stability under soil salinity conditions than other test entries. The significant advantage resulted in the accession M-2579, which achieved the maximum green mass yield of 138.0 t/ha (+7.5% over check). This level of productivity aligns with recent reports on the high genetic potential of local Kazakh varieties like 'Tozimdi,' developed to ensure reliable harvests despite increasing climate stress (Crop Trust, 2025). The leading genotypes' group also included accessions M-2513 (135.3 t/ha) and M-2587 and M-2588 (133.4 t/ha each), with increases over the check genotype ranging from 3.9% to 5.4%.

**Table 1.** Agro-biological indicators of alfalfa genotypes in the control nursery averaged over 2023–2025.

No.	Cultivar / Accessions	Leafi- ness (%)	Average plant Height (cm)	Total green mass yield over 5 cuttings (t/ha)	Percent of Standard	Total dry matter yield over 5 cuttings (t/ha)	Percent of Standrard	1000- seed weight (g)	Individual seed selections (g)
1	Damu-12-St.	49.0	82.6	128.4	100	20.6	100	2.5	10.7
2	M-2500	45.2	85.2	116.8	91.0	20.0	97.1	2.5	10.4
3	M-2501	46.2	85.8	117.2	91.3	20.5	99.5	2.3	9.7
4	M-2504	48.6	85.8	128.2	99.8	22.1	107.3	2.4	9.9
5	M-2509	44.8	84.6	129.3	100.7	20.8	101.0	2.7	12.3
6	M-2511	45.4	84.6	125.8	98.0	21.6	104.9	2.3	9.9
7	M-2513	45.0	80.6	135.3	105.4	22.4	108.7	2.1	12.0
8	M-2515	52.2	79.2	130.0	101.2	22.8	110.7	2.4	9.0
9	M-2518	47.2	82.8	120.4	93.8	22.0	106.8	2.3	9.0
10	M-2520	46.2	91.0	120.4	93.8	23.1	112.1	2.4	9.8
11	M-2521	47.2	87.8	134.1	104.4	23.1	112.1	2.2	9.9
12	M-2532	45.6	78.8	130.2	101.4	22.8	110.7	2.2	9.2
13	M-2533	49.6	89.2	133.2	103.7	20.8	101.0	2.4	9.6
14	M-2542	47.6	88.2	116.9	91.0	21.4	103.9	2.4	12.1
15	M-2561	51.2	96.6	128.5	100.1	20.8	101.0	2.6	10.2
16	M-2573	47.8	87.0	130.0	101.2	21.8	105.8	2.5	12.3
17	M-2579	50.2	84.8	138.0	107.5	22.8	110.7	2.5	8.6
18	M-2580	47.2	82.6	120.8	94.1	21.8	105.8	2.5	9.3
19	M-2581	44.6	83.6	126.4	98.4	21.8	105.8	2.6	9.8
20	M-2584	47.8	83.2	128.8	100.3	22.7	110.2	2.5	8.6
21	M-2587	47.6	79.8	133.4	103.9	22.9	111.2	2.5	10.3
22	M-2588	50.8	80.0	133.4	103.9	22.8	110.7	2.6	9.7
23	MS-1969	48.6	79.6	111.6	86.9	21.4	103.9	2.3	9.6
24	M-2582	46.2	92.0	128.4	100	22.4	108.7	2.7	10.1
25	M-2594	47.2	83.6	129.4	100.8	22.5	109.2	2.4	12.0
26	M-2553	49.6	89.6	132.4	103.1	20.8	101.0	2.3	12.1
27	M-2562	45.8	79.0	130.5	101.6	23.1	112.1	2.4	12.2
28	M-2572	47.2	80.2	131.9	102.7	23.8	115.5	2.4	12.3
29	M-2589	47.8	93.0	127.1	99	22.7	110.2	2.4	12.2

Note:  $LSD_{0.05} = 0.22$  t/ha (for dry matter yield).

Dry matter yield was considerably the most objective criterion for evaluating forage value. According to results, most studied accessions demonstrated the highest breeding values. The maximum dry matter yield was notable for the accession M-2572 (23.8 t/ha), which was significantly higher than the check cultivar by 3.2 t/ha (15.5%). Similar significant correlations between environmental factors and dry matter yield (DMY) in Central Asian regions recently a discussion from Zotova *et al.* (2024), confirming that drought-resistant traits are paramount for stable biomass production. Consistently, some other accessions, such as M-2520, M-2521, and M-2562, also showed

maximum productivity (23.1 t/ha and +12.1% over the check).

The analysis of alfalfa productivity in relation to hydrothermal conditions (2023–2025) revealed a strong dependence of regrowth rates on cumulative active temperatures. During the peak heat periods of 2024 (July–August), a slight reduction in plant height gained compensation from an increased leaf density in elite accessions (M-2515 and M-2579), indicating high adaptive plasticity. Despite the precipitation deficit, maintaining the 5-cutting cycle continued due to optimal irrigation scheduling, which mitigated the negative impact of extreme evaporative demand on dry matter accumulation.

### Morphological and biological yield structure

The morpho-biological analysis of the yield structure revealed that for leafiness, it is a key indicator of nutritional value. The alfalfa accessions M-2515 (52.2%) and M-2561 (51.2%) emerged as leading. High leaf-to-stem ratio is a critical selection criterion, as noted by Karychev *et al.* (2023), who found that leafiness directly correlates with protein content in alfalfa populations under semi-arid conditions. Biochemical analysis of the leading accessions disclosed high crude protein content, ranging from 21.4% to 21.8% in dry matter for M-2515 and M-2579, which significantly exceeds the standard (19.2%). This confirms that high leafiness (over 50%) in these genotypes directly correlates with superior forage quality. Additionally, the accession M-2561 exhibited the highest growth intensity, reaching an average plant height of 96.6 cm. Regarding the 1000-seed weight, which determines sowing quality, the best results (2.7 g) came from the accessions M-2509 and M-2582, exceeding the check cultivar by 8.0%. Meirman *et al.* (2020) reported similar variations in seed weight and quality for alfalfa varieties adapted to the saline soils of Southern Kazakhstan.

On the individual seed selections, the significant variations were evident among the accessions for this parameter. A group of alfalfa accessions (M-2509, M-2513, M-2542, and M-2594) showed minimum values (2.0–2.3 g), while most accessions ranged between 8.6 and 10.7 g. However, the highest values appeared for the check cultivar Damu-12 (10.7 g) and accession M-2500 (10.4 g).

### Correlation analysis

In the breeding process, to determine the effectiveness of an indirect selection, a correlation analysis took place between the primary quantitative and qualitative traits of the studied alfalfa genotypes (Table 2). The analysis stated a significant ( $p < 0.01$ ) positive correlation ( $r = 0.74$ ) between the green mass and dry matter yields. This interrelationship confirmed the highest heritability of biomass accumulation traits under the conditions of the Maktaaral District, allowing for effective selection for overall productivity.

A moderate positive correlation ( $r = 0.32$ ) was distinct between the leafiness and dry matter yield. The results revealed that in the studied alfalfa accessions (especially the leading genotypes M-2572 and M-2579), the increase in productivity occurs not only through stem lignification but also through the preservation of the leaf apparatus. This characteristic proved critically important for maintaining forage quality under arid environments.

A moderate negative correlation ( $r = -0.41$ ) was noteworthy between the plant height and leafiness in the control nursery. However, this relationship varied in the competitive nursery ( $r = 0.33$ ), suggesting that selection can effectively combine increased height with high leaf proportion. This indicates a biological trade-off where rapid stem elongation often leads to a lower leaf-to-stem ratio, yet advanced breeding lines can decouple these traits. Specifically, compromise genotypes, such as M-2588, are of particular interest for breeding, as they combine above-average height (80.0 cm) with high leafiness (50.8%),

**Table 2.** Correlation coefficients ( $r$ ) between economically valuable traits of alfalfa genotypes in the control nursery averaged over 2023–2025.

Traits	Dry matter yield	Plant height	Leafiness	1000-seed weight
Green mass yield	0.74**	0.28	0.15	−0.12
Dry matter yield	1	0.22	0.32*	−0.08
Plant height	—	1	−0.41*	0.18

\* — significant at  $P < 0.05$ ; \*\* — significant at  $P < 0.01$

ensuring both high biomass volume and superior forage quality. The weak correlation ( $r = -0.12$ ) between 1000-seed weight and vegetative mass yield revealed the possibility of autonomous improvement of these traits within a single population without significant detriment to either characteristic.

### Competitive varietal trial

Twenty alfalfa accessions succeeded in their evaluation in a competitive nursery against the standard cultivar Damu-12. All agrotechnical measures were identical to those applied in the breeding nursery. After the overwintering period, the winter hardiness of the alfalfa accessions entailed assessment in the competitive nursery. Results showed the highest winter hardiness across all the accessions, with 85%–95% of plants surviving

per one 1 m<sup>2</sup>. These findings align with the observations of Smith and Johnson (2021), who emphasized that high survival rates in competitive nurseries are critical indicators of regional adaptation for perennial legumes. Regarding the tillering capacity of tested accessions, 16 accessions exhibited the highest tillering capacity (62–65 stems), while five genotypes showed medium tillering (45–50 stems) (totaling 21 genotypes).

A detailed analysis of the alfalfa accessions progressed during 2023–2025 according to key economic and biological traits in comparison with the check cultivar Damu-12 (Table 3). Based on the evaluation of green mass productivity, the average yield across all accessions was 123.15 t/ha. Though the check cultivar Damu-12 (128.4 t/ha) showed high stability, several accessions, including the selection material, performed at a competitive

**Table 3.** Economic and biological parameters of alfalfa genotypes in the competitive variety trial averaged over 2023–2025.

No.	Cultivars / Accessions	Leafiness (%)	Average plant height (cm)	Total green mass yield over 5 cuttings (t/ha)	Percent of Standard	Total dry matter yield over 5 cuttings (t/ha)	Percent of Standard	1000-seed weight (g)	Individual seed selections (g)
1	Damu-12 (St.)	49.4	86.2	124.6	100	20.5	100	2.7	14.1
2	M-2572	44.6	99.2	134.5	107.9	23.1	112.7	2.7	14.2
3	M-2584	48.2	96.8	125.8	101.0	23.0	112.2	2.8	14.1
4	M-2518	47.8	84.2	120.4	96.6	21.8	106.3	3.0	14.3
5	M-2533	49.2	89.2	133.5	107.1	20.6	100.5	2.7	14.1
6	M-2532	44.4	79.4	130.9	105.1	23.1	112.7	2.5	14.0
7	M-2501	45.8	86.8	117.0	93.9	20.6	100.5	2.4	14.0
8	M-2568	48.6	84.8	115.0	92.3	22.0	107.3	2.5	14.0
9	M-2566	46.2	79.4	117.3	94.1	23.2	113.2	2.5	13.9
10	M-2537	47.8	95.4	125.6	100.8	23.6	115.1	2.3	14.2
11	M-2513	44.8	81.6	138.3	111.0	22.1	107.8	2.5	14.1
12	M-2522	50.0	82.2	119.7	96.1	22.6	110.2	2.4	14.3
13	M-2579	50.6	84.6	138.2	110.9	22.8	111.2	2.4	13.9
14	M-2552	47.2	94.2	108.9	87.4	25.2	122.9	2.6	12,0
15	M-2582	46.2	91.2	129.2	103.7	23.3	113.7	2.7	14.2
16	M-2581	49.4	93.6	126.9	101.8	23.2	113.2	2.6	14.3
17	M-2587	46.8	80.4	131.8	105.8	22.1	107.8	2.7	14.4
18	M-2553	51.2	88.8	116.2	93.3	20.6	100.5	2.7	14.1
19	M-2547	50.4	93.6	124.0	99.5	21.1	102.9	3.0	14.0
20	M-2545	54.2	94.6	107.4	86.2	24.8	121.0	2.7	13.9
21	M-2539	52.4	97.6	116.8	93.7	19.8	96.6	2.5	14.0

LSD<sub>0.05</sub> = 0.19 t/ha

**Table 4.** Correlation matrix of agronomic traits in alfalfa genotypes.

Traits	Leafiness	Plant height	Green mass	Dry matter	1000-seed weight
Leafiness	1	0.33	-0.44*	-0.17	0.14
Plant height	0.33*	1	-0.19	0.14	0.22
Green mass	-0.44*	-0.19	1	0.81**	-0.03
Dry matter	-0.17	0.14	0.81**	1	-0.14
1000-seed weight	0.14	0.22	-0.03	-0.14	1

level under saline conditions. Eight alfalfa accessions significantly exceeded the standard cultivar, with the leading performers being M-2513 (138.3 t/ha, +10.9% over St.) and M-2579 (138.2 t/ha, +10.9% over St.). In contrast, accessions M-2545 and M-2552 displayed the lowest values of green mass yield (86.2%–87.4% of the standard). Similar variations in biomass productivity among breeding lines came from Brown *et al.* (2022), noting that genetic potential significantly outweighs environmental factors in high-input nurseries. Regarding dry matter yield, most accessions (19 out of 21) outperformed the standard cultivar. The highest dry matter yield appeared for the accession M-2552 (25.2 t/ha), which is +22.9% over the standard, enunciating its superior forage value despite a lower volume of green biomass than the check cultivar.

Morphological characteristics disclosed the maximum leafiness was recorded in accession M-2545 (54.2%), which was 4.8% higher than the standard cultivar Damu-12. The accession M-2572 emerged as the tallest (99.2 cm), which contributed to the formation of the highest green mass yield (107.9% relative to the standard cultivar Damu-12). Regarding seed productivity, accessions M-2518 and M-2547 were outstanding by their large seed size (1000-seed weight of 3.0 g). All other alfalfa accessions occurred at par and even below the standard (2.7 g). The correlation analysis reflects the relationship between the primary economic and biological traits (Table 4).

### Selection strategy and clustering

Primary determinants of productivity under the existing conditions were plant height, dry matter accumulation, and leafiness. Despite

the extreme hydrothermal stress of 2023–2025, characterized by temperatures up to +45 °C, the elite accessions (M-2515 and M-2588) maintained a high leaf retention. This adaptation is ascribable to enhanced stomatal regulation and the ability to maintain leaf turgor under high evaporative demand, preventing premature leaf senescence and abscission during the peak summer periods. A high leaf-to-stem ratio (exceeding 50% in M-2515 and M-2588) not only contributes to the total green mass yield but also determines the nutritional value and protein content of the forage, making it a critical selection criterion for cultivars adapted to saline soils. Systematizing the breeding strategy had the breeding material categorized into three clusters using k-means and hierarchical clustering based on leafiness, plant height, and green and dry mass yields.

The defined population architecture was with three distinct functional groups. Cluster 1 represents the elite biomass producers, while cluster 2 serves as a strategic reservoir for forage quality and dry matter density. The concentration of the standard variety (No. 1) within the low-variability and cluster 3 highlighted the significant performance gap between the commercial check and the identified high-potential lines.

Hierarchical clustering revealed a distinct divergence of intensive-type accessions from the general population. The core of cluster 1, as defined by the alfalfa accessions M-2513 and M-2579, formed a separate branch due to a unique parity of morphotypes and extreme green mass yield (> 138.4 t/ha) (Table 5). The localization of the standard cultivar Damu-12 within the low-variability and the cluster 3 justified the urgent need for cultivar replacement using elite lines from the first and second groups.

**Table 5.** Clustering of alfalfa accessions based on key agro-morphological traits.

Cluster	Group descriptor	Representative accessions (No.)
Cluster 1	Intensive: Maximum green mass yield, superior plant height, moderate leafiness.	M-2572 (2), M-2533 (5), M-2513 (11), M-2579 (13), M-2587 (17)
Cluster 2	High-Quality: Leaders in dry matter yield and leaf-to-stem ratio; moderate green mass.	M-2552 (14), M-2545 (20), M-2582 (15), M-2581 (16), M-2522 (12), M-2537 (10)
Cluster 3	Stable/Inert: Average to low performance across all traits; includes the check cultivar.	Damu-12 (1), M-2584 (3), M-2518 (4), M-2532 (6), M-2501 (7), M-2568 (8), M-2566 (9), M-2553 (18), M-2547 (19), M-2539 (21)

### Selection value

**Cluster 1 (High-Yield):** Primary resource for hay-type cultivars, with maximum efficiency in transforming environmental resources into vegetative biomass.

**Cluster 2 (Concentrated):** Sources of high dry matter content (leading accession: M-2552, 121.2% vs. the control) and drought resistance. These forms serve as essential donors for forage quality.

### Hybridization strategy

Breeding programs should focus on crossing contrastive genotypes from Cluster 1 and Cluster 2 to trigger a transgressive effect. The recommended accessions pair was M-2513 (growth energy) × M-2552 (dry matter). This combination design will converge rapid biomass accumulation with enhanced nutritional values in the progeny.

### CONCLUSIONS

Based on a comprehensive evaluation in the control nursery of alfalfa (*M. sativa* L.), the most promising accessions were M-2572, M-2579, and M-2521. The results of the competitive varietal trial demonstrated the selected alfalfa accessions significantly outperformed the check cultivar Damu-12 in total green mass and dry matter yields. According to the competitive varietal trial, the analyzed alfalfa accessions significantly outperformed the check cultivar Damu-12 in total dry mass yield. Accessions M-2572, M-2520, M-2521, M-2562, M-2515, and M-2579

were recognizably the best for their complex of economic and biological traits, including high leafiness (M-2515) and optimal plant height (M-2561). Accessions M-2572, M-2513, and M-2579 significantly excelled the check cultivar in total yield. The localization of the standard cultivar Damu-12 within the low-variability cluster (Cluster 3), while the elite lines achieved a maximum yield of 138.4 t/ha, justifies the urgent need for cultivar replacement using promising lines from the first and second clusters. In achieving a transgressive effect in hybridization, this study highly recommends crossing contrasting forms from Cluster 1 (M-2513) and Cluster 2 (M-2552) to combine the highest growth vigor with increased dry matter content in the offspring.

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