

SABRAO Journal of Breeding and Genetics
 58 (5) 2348-2356, 2026
<http://doi.org/10.54910/sabrao2026.58.5.34>
<http://sabraojournal.org/>
 pISSN 1029-7073; eISSN 2224-8978



DEVELOPMENT OF HIGH-YIELDING ALFALFA (*MEDICAGO SATIVA* L.) HYBRID WITH IMPROVED BIOMASS AND QUALITY UNDER AGROECOLOGICAL CONDITIONS OF UZBEKISTAN

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SUMMARY

The development of a high-biomass alfalfa (*Medicago sativa* L.) hybrid was the aim of this research, along with evaluating its agronomic and morphological performance under Uzbekistan conditions. The hybrid line Tashkent-3651 reached its development through conventional hybridization between the parental genotypes k-6365 (Sudan) and k-3189 (Asia Minor). The newly developed alfalfa hybrid (Tashkent-3651) and its parental cultivars (k-6365 and k-3189) reached assessment in comparison with the check genotype Tashkent-1 under field conditions. The results revealed significant ($p \leq 0.05$) differences among the genotypes for green biomass yield, hay yield, plant height, and leafiness. The hybrid Tashkent-3651 enunciated superior performance for higher green biomass and hay yield, as well as improved morphological traits compared with the standard cultivar Tashkent-1 and the parental genotypes. The increased productivity of the alfalfa hybrid showed an association with enhanced growth vigor, higher leafiness, and improved biomass accumulation. Overall, the results confirmed that hybridization between genetically diverse genotypes was an effective approach for improving biomass productivity and forage quality in alfalfa. The newly developed hybrid Tashkent-3651 showed the high potential for cultivation under agroecological conditions of Uzbekistan.

Communicating Editor: Dr. Anita Restu Puji Raharjeng

Manuscript received: April 17, 2026; Accepted: June 28, 2026.

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Citation: Khudoyberdiev NKH, Amanturdiev SHB, Toreev FN, Karimov RA, Karimova NA, Tangirova GN (2026). Development of high-yielding alfalfa (*Medicago sativa* L.) hybrid with improved biomass and quality under agroecological conditions of Uzbekistan. *SABRAO J. Breed. Genet.* 58 (5) 2348-2356. <http://doi.org/10.54910/sabrao2026.58.5.34>.

Keywords: Alfalfa (*M. sativa* L.), hybridization, biomass yield, hay yield, morphological traits, leafiness, Uzbekistan conditions

Key findings: The alfalfa (*M. sativa* L.) hybrid Tashkent-3651 (k-6365 × k-3189) significantly outperformed the standard cultivar and parental genotypes in biomass and hay yield. The hybrid higher plant height (95 ± 2.4 cm) and leafiness ($49.2\% \pm 1.2\%$) considerably contributed to the improved forage productivity and quality.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important perennial leguminous forage crops worldwide. Its cultivation occurs in more than 80 countries on over 40 million hectares due to its highest biomass productivity, superior nutritional values, and generous adaptability to diverse agroecological conditions (Koptileuov *et al.*, 2024). As a representative of the family Leguminosae, the genus *Medicago* includes numerous species with wide recognition as an effective biological ameliorant, improving soil fertility through biological nitrogen fixation and suppressing soil-borne pathogens (Kvitko, 1990).

In Uzbekistan and other Central Asian regions, alfalfa plays a crucial role in sustainable agricultural systems, particularly under arid and semi-arid conditions. It considerably contributes to maintaining soil fertility, enhancing nitrogen balance, and supporting the livestock production sector. The inclusion of perennial legumes, such as alfalfa, in crop rotation systems has been prevalent in gently improving the soil organic matter and humus content, thereby enhancing the productivity of subsequent crops (Meliev *et al.*, 2023).

The alfalfa's high feeding value can refer to its rich biochemical composition, including proteins, essential amino acids, vitamins, and mineral elements. Alfalfa is particularly rich in carotenoids and vitamins B, C, D, E, and K, as well as important minerals, such as calcium and phosphorus, proving it as a highly valuable forage crop for livestock production. Moreover, alfalfa contains biologically active compounds, such as saponins and tannins, which enhance the immune system and improve animal health

(Koptileuov *et al.*, 2024). These properties identified alfalfa as not only a high-quality feed crop but also a plant with functional and medicinal importance.

Despite its numerous advantages, alfalfa production often suffers limitations from various abiotic stress conditions, such as drought, salinity, and high temperature, as well as biotic stresses, including pests and diseases. These constraints become more severe in regions with environmental challenges, such as the Aral Sea basin, where soil salinization, water scarcity, and land degradation considerably reduced the overall productivity of crops (Lancefield *et al.*, 2009). Therefore, the development of high-yielding and stress-adapted alfalfa cultivars remains a key priority for sustainable forage production.

Conventional alfalfa breeding is mainly dependent on the hybridization and phenotypic selection. Given its autotetraploid nature and high genetic variability, alfalfa enunciates considerable genetic diversity for morphological and agronomic traits (Myrzabek, 1998). Therefore, hybridization between genetically distinct parental genotypes allows the expression of heterosis, resulting in improved biomass yield and adaptability. In practical breeding programs, the selection of promising genotypes relied primarily on field evaluation of agronomic traits, particularly biomass productivity, growth intensity, and adaptation to existing environmental conditions (Adhikari and Missaoui, 2017).

Previous studies revealed perennial legumes, especially alfalfa, considerably contribute to soil nutrient accumulation and agricultural sustainability. For instance, alfalfa can accumulate substantial amounts of biological nitrogen and organic residues, improving soil structure and fertility over time

(Bastaubayeva *et al.*, 2023). Moreover, the integration of alfalfa into crop rotation systems enhances overall farm productivity and reduces the dependency on mineral fertilizers (Osterholz *et al.*, 2020).

Despite these advances, a critical gap still exists in developing new alfalfa genotypes combining high biomass productivity with adaptation to existing environmental stresses. Particularly under Uzbekistan's agroecological conditions, the development of new high-yielding hybrids through the hybridization of selected parents holds greater scientific and practical importance. Therefore, the presented study aimed to develop a new high-yielding alfalfa hybrid through the hybridization of selected parental genotypes and identify promising genotypes based on biomass productivity under Uzbekistan's environmental conditions.

MATERIALS AND METHODS

Plant material

The study began using selected alfalfa (*M. sativa* L.) genotypes, including one newly developed hybrid line (Tashkent-3651), both of its parental genotypes (k-6365 and k-3189), and one standard cultivar (Tashkent-1). Advancing the hybrid population continued through successive generations, with the superior line selected from the F6 generation subsequently released as the cultivar Tashkent-3651 based on its favorable agronomic performance. The hybrid line development proceeded through crossing between the parental genotypes k-6365 (Sudan) and k-3189 (Asia Minor). The resulting hybrid population entailed advancement through subsequent selection cycles. The breeding process involved phenotypic selection aimed at identifying superior genotypes with enhanced biomass productivity and adaptability to prevailing environmental conditions (Erjigitov *et al.*, 2025).

Field experiment

The field experiments took place at the experimental sites of the Research Institute of Cotton Breeding, Seed Production, and Agrotechnologies under the agroecological conditions of Uzbekistan. The studies transpired during the period of 2018–2025. The climate features of the study site were arid to semi-arid, with high summer temperatures and limited water availability, which considerably influenced the growth and productivity of forage crops. The experimental soils were typical for irrigated agricultural zones of Uzbekistan, with moderate fertility and varying levels of salinity, which are common constraints affecting alfalfa production under existing conditions. The field experiment layout was in a randomized complete block design with three replications. The experimental plots had random assignments within each block to minimize environmental variation. Growing all genotypes was under identical agronomic conditions throughout the experimental period.

Data collection and statistical analysis

All these four alfalfa genotypes received evaluation through comparative analysis of plant growth and productivity traits under field conditions during the growing season. Data recording included plant height, leafiness growth rate, yield-related traits (green biomass and hay yield), and protein content. Obtaining green biomass yield resulted from harvesting plants at the flowering stage. Fresh biomass from each plot sustained weighing immediately after cutting, expressed as $t\ ha^{-1}$. Hay yield determination was after natural open-air drying of the harvested biomass until achieving the constant weight, with the dry biomass then converted into $t\ ha^{-1}$. The obtained data underwent assessment using the analysis of variance (ANOVA) performed with the OriginPro 2021 software package. The analysis sought to determine the significance of differences among the genotypes and evaluate the superiority of the hybrid line compared with the standard cultivar (Turaev *et al.*, 2023).

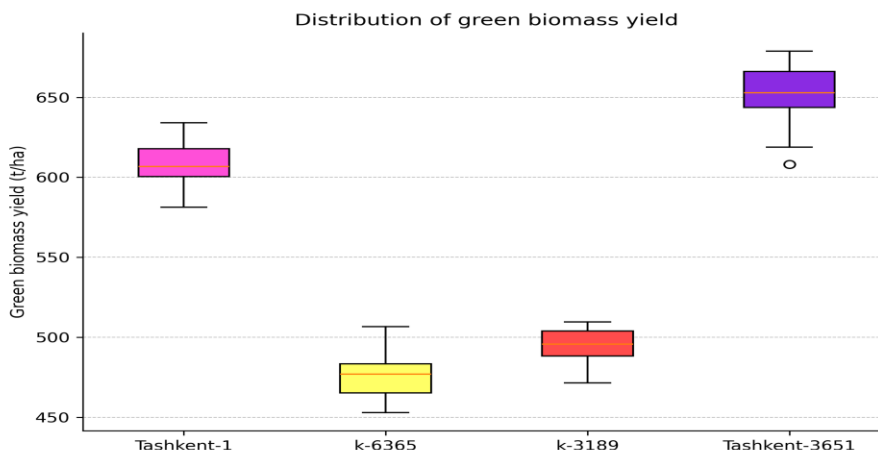


Figure 1. Variability among the standard, parental, and hybrid genotypes of alfalfa for green biomass yield (t/ha).

RESULTS AND DISCUSSION

The evaluation of alfalfa's (*M. sativa* L.) newly developed hybrid line, its parental genotypes, and the check cultivar revealed significant differences for biomass productivity, morphological traits, and forage quality under the agroecological conditions of Uzbekistan. The results clearly confirmed the effectiveness of hybridization and phenotypic selection in improving economically valuable traits of the alfalfa.

Green biomass yield

In forage crop evaluation, green biomass yield is a key indicator as it directly determines the feed availability and productivity. In this study, the hybrid line Tashkent-3651 showed superior performance compared to both the parental genotypes and the standard cultivar (Figure 1). The average annual green biomass yield of Tashkent-3651 ranged from 531.7 ± 15.2 to 773.2 ± 18.6 t/ha, while the check cultivar Tashkent-1 produced 507.2 ± 13.8 to 713.2 ± 16.4 t/ha. The parental genotypes (k-6365 and k-3189) also exhibited relatively lower green biomass yields (480.5 ± 14.1 and 495.3 ± 13.5 t/ha, respectively).

The superiority of the hybrid line over both the parental genotypes and the standard cultivar revealed the primary role of heterosis,

which significantly enhanced biomass accumulation. Previous studies also reported that hybridization in alfalfa genotypes resulted in increased biomass yield and improved agronomic performances due to the cross combination of genetically diverse parental forms (Bastabayeva *et al.*, 2023).

In the hybrid line Tashkent-3651, the observed increase in biomass yield (5%–10%) compared with the standard cultivar was consistent with earlier reports revealing hybrid vigor contributes to enhanced physiological efficiency, including improved photosynthesis and nutrient utilization (Kalin *et al.*, 2025). Moreover, the results showed alfalfa genotypes originating from stress-prone regions, such as Sudan, often possess traits related to drought and salinity tolerance, which can significantly enrich hybrid performance under arid conditions.

In agreement with the present results, several studies have demonstrated that the integration of genotypes with diverse genetic backgrounds leads to improved adaptability and productivity in forage crops, particularly under unfavorable environmental conditions (Toktarbekova *et al.*, 2020). These studies emphasized that hybrid genotypes maintained their higher productivity due to better stress tolerance mechanisms and stable growth dynamics.

Table 1. Variability among the standard, parental, and hybrid genotypes of alfalfa for hay yield (t/ha).

Genotypes	Means (t/ha)	SD	Mean $\pm$ SD (t/ha)	CV (%)	S.E.	Group	Increase vs. standard (%)
Tashkent-3651	159.2	5	159.2 $\pm$ 5.0	3.14	1.58	a	9.11
Tashkent-1	145.9	4.5	145.9 $\pm$ 4.5	3.08	1.42	b	-
k-3189	121.2	3.8	121.2 $\pm$ 3.8	3.13	1.20	c	-16.92
k-6365	118.4	3.6	118.4 $\pm$ 3.6	3.04	1.13	c	-18.8

Note: CV (%) = coefficient of variation; SD = standard deviation; S.E. = standard error.

Hay yield

Hay yield is also one of the most crucial agronomic and economic indicators in alfalfa production, as it directly reflects the amount of usable dry matter available for livestock feeding. Unlike green biomass, hay yield provides a more reliable measure of productivity because it accounts for moisture loss and directly represents nutritive values of forage under storage and feeding conditions. The presented green biomass and hay yield values represent the total annual productivity accumulated across all harvests during the growing season. Therefore, improving hay yield is a primary objective in alfalfa breeding programs aimed at enhancing feed efficiency and economic returns.

In the concerned study, significant differences were evident among the evaluated alfalfa genotypes for hay yield (Table 1). The hybrid line Tashkent-3651 enunciated the superior performance compared with both the standard cultivar and parental genotypes. In the hybrid line Tashkent-3651, the hay yield ranged from 129.7 $\pm$ 4.2 to 188.6 $\pm$ 5.8 t/ha, whereas the check cultivar Tashkent-1 emerged with 123.7 $\pm$ 3.9 to 174.0 $\pm$ 5.1 t/ha. The parental genotypes k-6365 and k-3189 showed relatively lower yields (118.4 $\pm$ 3.6 and 121.2 $\pm$ 3.8 t/ha, respectively). The results revealed that the hybrid line exceeded the standard cultivar by 4%–8%, confirming its improved dry matter productivity. This increase is particularly valuable under the agroecological conditions of Uzbekistan, where efficient forage utilization is essential due to limited water resources and high evaporation rates. The promising results were consistent with previous findings reported in alfalfa breeding research. For instance, Asanov *et al.* (1990) emphasized that hybridization can

significantly improve both yield and persistence in alfalfa due to heterosis.

In the hybrid line Tashkent-3651, the higher hay yield can be due to improved vegetative growth and a higher proportion of leaf biomass. Plant leaves are the main contributors to dry matter accumulation and contain a higher concentration of nutrients than in stems. The hybrid line exhibited increased leafiness and more efficient biomass partitioning, which eventually resulted in greater dry matter accumulation. Another important factor contributing to increased hay yield is the remarkable regrowth ability after cutting. In multi-harvest systems, which are typical for alfalfa cultivation in Uzbekistan, the capacity of plants to rapidly regenerate after each cut determines the total annual green and dry biomass yield. The hybrid line Tashkent-3651 maintained the stable regrowth across successive cuts, revealing enhanced physiological activity and energy reserve utilization. Similarly, Cui *et al.* (2018) reported hybrid genotypes exhibit higher dry matter yield as a result of improved growth vigor and biomass accumulation.

The higher hay yield in the hybrid line Tashkent-3651 suggested that this genotype may possess improved structural and physiological characteristics that reduce dry matter loss during harvesting and drying processes. Furthermore, Rimi *et al.* (2010) highlighted that efficient biomass accumulation and reduced losses during drying occurred to be crucial factors influencing hay yield. In agreement with Absattar *et al.* (2015) and Kalin *et al.* (2023), the development of high-yielding and stress-tolerant alfalfa genotypes is particularly essential under arid and semi-arid conditions. The superior performance of the hybrid line Tashkent-3651 displayed that the hybrid successfully combines the adaptive

traits of its parental forms, resulting in improved productivity under existing environmental constraints.

Morphological traits

Morphological traits, particularly plant height and leafiness, were the key indicators of growth potential and forage quality in alfalfa, as these traits directly influenced light interception, biomass accumulation, and nutrient composition. In the presented study, the apparent differences were notable among the evaluated genotypes for plant height and leafiness, reflecting their genetic diversity and adaptive potential in the existing environment.

The hybrid line Tashkent-3651 exhibited significantly higher plant height (95 ± 2.4 cm) compared with the standard cultivar Tashkent-1 (76 ± 2.3 cm) and parental genotype k-6365 (68 ± 2.1 cm), while slightly lower than the second parental genotype k-3189 (98 ± 3.1 cm) (Figure 2). Similarly, the hybrid showed the highest leafiness ($49.2\% \pm 1.2\%$) compared with the check cultivar Tashkent-1 ($45.5\% \pm 1.7\%$) and both parental genotypes k-6365 ($41.0\% \pm 1.3\%$) and k-3189 ($43.0\% \pm 1.4\%$). In the hybrid line Tashkent-3651, the observed increase in plant height and leafiness revealed improved growth vigor and more efficient biomass formation.

Taller plants generally have a greater capacity for light interception and photosynthetic activity, which enhances biomass accumulation in crop plants. However, the present hybrid did not exceed the tallest genotype (k-3189), suggesting a balanced inheritance of traits that combines sufficient height with structural stability, thereby reducing the risk of lodging. Moreover, Yuegao *et al.* (2009) also emphasized that an optimal balance between plant height and leaf-to-stem ratio is essential for maximizing both yield and forage quality.

The higher leafiness in the hybrid Tashkent-3651 was particularly important from a forage quality perspective, as leaves contain higher concentrations of protein and digestible nutrients than stems. The increased leaf proportion in the hybrid suggested improved biomass partitioning toward nutritionally valuable plant components, reflecting enhanced physiological efficiency and better assimilate allocation. In the hybrid line Tashkent-3651, the integrated improvement of plant height and leafiness can be because of the heterotic effects resulting from hybridization between genetically diverse genotypes (k-6365 and k-3189). Rimi *et al.* (2010) and Meirman *et al.* (2026) also reported that hybridization in genetically diverse genotypes considerably enhanced morphological and agronomic traits in alfalfa.

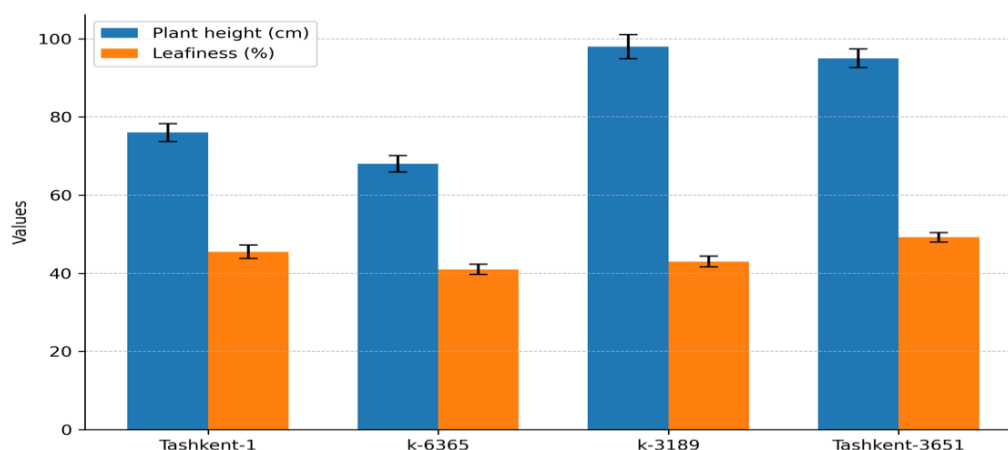


Figure 2. Distribution of plant height (cm) and leafiness (%) among the alfalfa genotypes using grouped boxplots.

In this context, the hybrid line Tashkent-3651 demonstrated a favorable plant architecture, combining moderate-to-high plant stature with increased leafiness, which considerably contributes to improved canopy structure and efficient light utilization. The hybrid line's ability to sustain higher leafiness and adequate plant height revealed improved adaptability under abiotic stress conditions. Under the agroecological conditions of Uzbekistan, characterized by high temperatures and limited water availability, such morphological traits proved particularly vital for maintaining stable productivity (Norboyeva *et al.*, 2025).

Forage quality

In alfalfa production systems, the forage quality is a critical factor, as it directly determines the nutritional values of the feed and its efficiency in livestock production. Among forage quality traits, protein content and fiber composition are particularly crucial, which influence the digestibility, feed intake, and overall animal performance. Therefore, improving forage quality is one of the main objectives in alfalfa breeding programs. In the relevant study, significant variations were evident among the evaluated alfalfa genotypes for protein content. The hybrid line Tashkent-3651 showed the highest protein content ($19.4\% \pm 0.55\%$), which was considerably higher than the standard cultivar Tashkent-1

($17.8\% \pm 0.4\%$) and parental genotype k-3189 ($16.3\% \pm 0.73\%$) while comparable with the second parental genotype k-6365 ($19.9\% \pm 0.45\%$) (Figure 3).

In the hybrid line Tashkent-3651, the relatively higher protein content revealed an improved forage quality and suggested enhanced metabolic activity and nitrogen assimilation. This improvement can be in association with increased leafiness in the hybrid, as leaves contain a higher concentration of protein than stems. In addition to protein content, the hybrid line exhibited slightly lower fiber content than the check cultivar, which also confirmed the better digestibility and higher nutritional value. Lower fiber content facilitates improved feed intake and nutrients' absorption in livestock. In alfalfa, the hybridization leads to improvements in biomass yield, as well as forage quality parameters such as protein content and digestibility (Kalin *et al.*, 2025).

Concerning the hybrid line Tashkent-3651, the observed improvement in forage quality traits can be ascribable to heterosis effects resulting from hybridization between genetically diverse parental genotypes (k-6365 and k-3189). The parental genotype k-6365 likely contributed higher protein potential, while k-3189 may have contributed to structural balance and plant stability. The integration of these valuable traits resulted in a hybrid with improved yield and quality traits. Furthermore, Cui *et al.* (2018) emphasized that

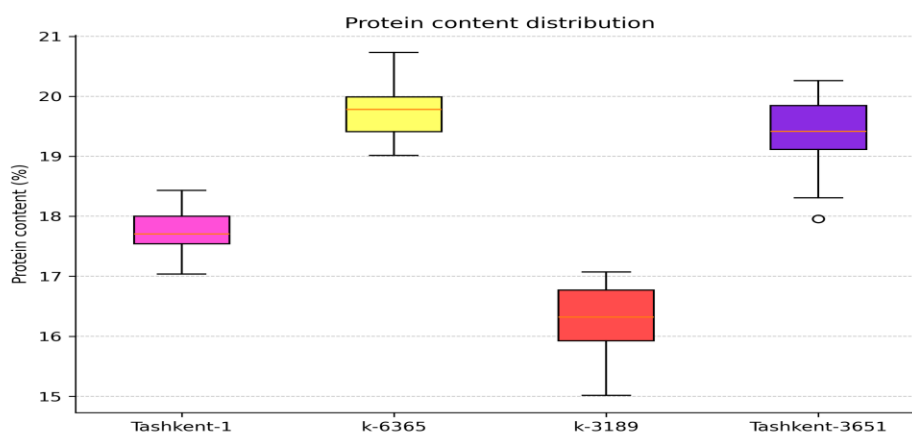


Figure 3. Variability among the alfalfa genotypes for protein content (%).

increased leaf proportion and reduced fiber content were the key factors contributing to higher forage quality.

Overall, the results enunciated that the hybrid line Tashkent-3651 possesses improved forage quality traits compared with the standard cultivar and one of the parental genotypes. The combination of higher protein content and lower fiber content indicated that this hybrid is a promising genotype for improving livestock productivity under Uzbekistan conditions.

CONCLUSIONS

The results revealed hybridization of genetically diverse alfalfa (*M. sativa* L.) genotypes was an effective approach for developing high-yielding and adaptable cultivars under Uzbekistan conditions. The hybrid Tashkent-3651 (k-6365 × k-3189) showed superior performance compared with the standard cultivar Tashkent-1. The hybrid also exhibited improved biomass yield and key morphological traits, contributing to enhanced forage productivity. The observed differences among the alfalfa genotypes were significant ($p \leq 0.05$), confirming the reliability of results. The superior performance of the hybrid line has a likely association with heterotic effects and the combination of desirable traits in parental qualities. Overall, the alfalfa hybrid Tashkent-3651 can be recognizably a promising genotype for future breeding programs and large-scale cultivation.

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