



## Development of Stress-resistant Sweet Clover (*Melilotus adans.*) Cultivars in Northern Kazakhstan

Nadezhda Filippova\*, Evgeniy Parsayev, Tatiana Kobernitskaia, Irina Rukavitsina, Irina Chilimova and Svetlana Dashkevich

A.I. Barayev Research and Production Centre for Grain Farming, Nauchny, Akmola Region, Kazakhstan

\*Corresponding author: [filippova-nady@mail.ru](mailto:filippova-nady@mail.ru)

### ABSTRACT

The study aimed to develop stress-resistant *Melilotus* (sweet clover) cultivars to mitigate protein deficiency in livestock feed and enhance forage productivity under the challenging agro-climatic conditions of Northern Kazakhstan, encompassing zonal (chernozem, chestnut) and saline soils. The research utilized diverse genetic resources, including accessions from the N.I. Vavilov Institute of Plant Genetic Resources, hybrid populations, and both domestic and foreign cultivars. Breeding efforts combined selection and hybridization methods to obtain genotypes with improved drought, winter, and disease resistance. Salt tolerance was evaluated using a modified laboratory protocol with 1.05% NaCl solution, followed by multi-year field trials conducted on sulfate-chloride saline steppe soils. Among the developed cultivars, *Melilotus wolgicus* 'Bars' and 'Qarlybas' exhibited high stress tolerance and superior agronomic performance, producing green mass yields of 19.4–22.8t  $ha^{-1}$ —14.7–22.2% higher than the standard—and crude protein contents of 19.4–20.1%. Salt-tolerant accessions showing 62–87.5% germination under salinity included the yellow sweet clover 'Sarbas' and hybrid populations (kd-1728, kd-1690, kd-1828, kd-1830, etc.). The most promising hybrid, 'Aq tań' (kd-1829), demonstrated 85% salt tolerance, 94% first-year survival, and high yield stability on saline soils, producing 13.4t  $ha^{-1}$  of green mass (+16.1%), 3.5t  $ha^{-1}$  of dry matter (+16.0%), and 170kg  $ha^{-1}$  of seeds (+14.7%) relative to the standard. The newly developed cultivars—'Bars', 'Qarlybas', and 'Aq tań'—significantly enhance forage productivity, protein content, and environmental resilience in Northern Kazakhstan. These genotypes represent sustainable breeding achievements that can support livestock production systems on saline and marginal lands while contributing to regional feed self-sufficiency.

### Article History

Article # 25-599

Received: 02-Oct-25

Revised: 18-Nov-25

Accepted: 27-Nov-25

Online First: 26-Dec-25

**Keywords:** Sweet clover, *Melilotus*, Breeding, Cultivar, Salt tolerance, Hybrid population, Yield, Green mass, Dry matter, Crude protein.

### INTRODUCTION

Selective breeding of annual and perennial forage crops is one of the key goals in feed production systems. In order to enable the establishment of livestock farming and mitigate protein deficiencies in animal feed, one key focus is the enhancement of the yield of protein-rich forage through the extension of the production of perennial leguminous grasses (Starodubtseva, 2014). Use of high-yielding species and cultivars that are well adapted to specific agro-climatic conditions is a key biological principle to break production constraints in forage. This will result in productive and sustainable longevity, the

production of high-protein and energy-dense feed, and the attainment of optimum environmental factors (Kosolapov et al., 2015; Pilipko, 2017). Introduction of new and more productive cultivars in respective sites can raise forage productivity by 20–70% and significantly enhance feed production (Shatsky et al., 2016; Kosolapov et al., 2016; Sagalbekov, 2018; Mu, 2021). Sweet clover (*Melilotus Adans.*) is a key indicator to increase the effectiveness of feed production in southern carbonate chernozems, chestnut soils, and sodic soils of the arid steppes. Sweet clover is a member of the *Fabaceae* family and includes 26 Eurasian and North African annual and biennial species, all included in the genus *Melilotus* Adans (Aboel-Atta, 2009).

**Cite this Article as:** Filippova N, Parsayev E, Kobernitskaia T, Rukavitsina I, Chilimova I and Dashkevich S, 2026. Development of stress-resistant sweet clover (*Melilotus adans.*) cultivars in northern Kazakhstan. International Journal of Agriculture and Biosciences 15(2): 719-726.  
<https://doi.org/10.47278/journal.ijab/2025.229>



A Publication of Unique Scientific Publishers

The genus is divided into three subgenera: Asian, Caspian, and Mediterranean. The most agronomic value is represented by the Asian subgenus, which includes yellow (or medicinal) sweet clover (*M. officinalis* (L.) Pall.), white sweet clover (*M. albus* Medic.), fragrant sweet clover (*M. suaveolens* Ledeb.), and toothed sweet clover. The Caspian subgenus is represented by the Volga sweet clover (*M. wolgicus* Poir.). Six species of sweet clover are native to Kazakhstan (Baitenov et al., 1969), among which biennial ones—yellow, white, Volga, and fragrant sweet clover—are cultivated most widely. Sweet clover (*Melilotus* spp.) is a valuable leguminous crop widely recognized for its dual role as a high-quality forage and an efficient green manure species that contributes substantially to soil fertility enhancement (De Dios Guerrero-Rodriguez et al., 2011; Aleksashina & Makarova, 2019). Its cultivation markedly improves the biological, agrophysical, and chemical properties of soils, enhances their phytosanitary condition, and effectively suppresses weed growth (Dzyubenko et al., 2018). In addition to its agronomic benefits, sweet clover demonstrates exceptional adaptability to adverse environmental conditions. Several studies have highlighted its potential as a phytoremediation crop capable of growing successfully on various salt-affected soils, thereby improving soil structure and fertility through biological nitrogen fixation and organic matter enrichment (Evans & Kearney, 2003; Sagalbekov & Ualieva, 2016; Parsayev & Filippova, 2017; Filippova et al., 2018). Among the two major cultivated species, white and yellow sweet clover, both exhibit high productivity in forage biomass. However, yellow sweet clover is particularly noted for its superior drought tolerance, making it better suited to regions experiencing dry spells during May and June (Parsaev, 2016). The harsh continental climate of Northern Kazakhstan, characterized by long winters, periodic droughts, and extensive areas of solonetz and saline soils (exceeding 16 million hectares), necessitates the development of winter-hardy, drought-resistant, and salt-tolerant sweet clover cultivars with improved agronomic and adaptive traits (Kazarina et al., 2023). The present study was undertaken to:

1. Develop new sweet clover (*Melilotus*) cultivars with enhanced tolerance to drought, salinity, and low temperatures suitable for the agro-ecological conditions of Northern Kazakhstan.
2. Evaluate the agronomic performance of newly developed cultivars in terms of forage biomass, seed yield, and protein content under both zonal (chernozem, chestnut) and saline soils.

Identify genetically stable and stress-resilient genotypes that can serve as productive and sustainable forage sources while improving the fertility and biological activity of degraded soils.

## MATERIALS & METHODS

### Study Area and Research Objects

The research on sweet clover (*Melilotus* spp.) was conducted in Northern Kazakhstan in the experimental crop rotation system of the A.I. Barayev Scientific

Production Center for Grain Farming (Scientific Village, Shortandy District, Akmola Region) for a prolonged period from 1972 to date. The subjects of research were an extensive collection of sweet clover genetic resources, including accessions of the N.I. Vavilov Institute of Plant Genetic Resources (VIR) world collection, complex hybrid populations (CHP), biotypes, breeding lines, and local and foreign cultivars.

### Material Selection

The breeding program adhered to the established methodological recommendations for perennial grasses (Ivanovich, 2023). The first step was the meticulous selection of superior accessions (k-35986, k-33613, k-37000) based on thorough screening procedures that assessed several agronomic characteristics. These included drought tolerance, plant height characteristics, leafiness indices, flowering and seed ripening synchronization trends, and overall seed production. Salt tolerance was evaluated as a critical component of selection with 90 accessions and CHPs subjected to laboratory germination testing in 1.05% NaCl solution following a modified VIR protocol (Sinelnikova et al., 1989). Systematic screening allowed for the classification of materials into five different salt-tolerance classes, from very low to high tolerance. Later, promising candidates with salt tolerance were planted in single field plots for further evaluation.

### Experimental Process

The experimental design involved both laboratory analysis and field research to ensure a careful assessment of plant material. Laboratory operations included screening for salt tolerance and comprehensive biochemical testing, particularly measurement of crude protein and fiber content in dry matter. Field experiments used the competitive variety testing (CVT) method, with test plots on weakly saline soils (sulfate-chloride salinity) and control southern carbonate chernozem plots. The heavy loams of chernozem-type soils contained a satisfactory 3.0-3.2% humus in the arable layer (Dashkevich et al., 2018; Filippova et al., 2022). Field tests placed emphasis on certain crucial parameters: resistance to abiotic stresses, which include winter hardiness and drought tolerance; susceptibility to major diseases such as Fusarium wilt, powdery mildew, and brown spot; and tolerance to pest damage caused by seed weevils (*Tychius*) and clover seed chalcid.

### Testing Protocols

Standardized test protocols generated consistency and reliability of data for all tests. Salt tolerance was evaluated by using an accelerated germination assay with a 1.05% NaCl solution, which was adapted from the original protocol utilized by VIR in testing amaranth. Biochemical assays gave consistent measurements for crude protein, fiber, and dry matter feed units. Statistical analysis employed a five-step salt tolerance scale in 20% increments, with particular note taken of plant responses at critical growth stages. Assessment of disease and pest resistance employed incidence scoring systems for the uniform measurement of susceptibility to fungal disease

and insect damage to seed.

### Breeding Achievements

The breeding program has some significant success, breeding 19 perennial legume cultivars to date, including eight sweet clover cultivars with four officially registered in the State Register of Breeding Achievements Recommended for use in the Republic of Kazakhstan (2023). Current breeding work maintains traditional objectives of breeding stress-tolerant hay-type cultivars with 12-15% yield advantage over existing standards, as well as pursuing new directions. These comprise the creation of salt-tolerant varieties with increased biomass yield, enhancement of nitrogen-fixing ability, and the creation of low-coumarin variants for forage quality improvement. The program strategy ideally integrates classical selection methods with contemporary stress-tolerance test technologies, while the two-stage validation system (laboratory and field assessment) guarantees ecological relevance and applicability of the outcomes. The explicit connection between strict methodological frameworks (like the adapted VIR protocols) and tangible outcomes in breeding has been a defining feature of this extensive research project.

### Statistical Analysis

All experimental data were subjected to statistical evaluation to ensure the reliability and reproducibility of results. Laboratory and field experiments were carried out in a completely randomized block design (CRBD) with three replications for each treatment or cultivar. Statistical analysis was performed using STATISTICA 12.0 (StatSoft Inc., USA) and Microsoft Excel 2021 for initial data organization and descriptive statistics.

### Data Processing and Model

The mean values for each trait—germination percentage, survival rate, green mass yield, dry matter yield, seed yield, and crude protein content—were computed for all cultivars and hybrid populations. The following model was applied for analysis of variance (ANOVA):

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

where  $Y_{ij}$  is the observed value of the  $i^{th}$  treatment (cultivar or hybrid) in the  $j^{th}$  replication,  $\mu$  is the overall mean,  $T_i$  represents the fixed effect of treatment, and  $\epsilon_{ij}$  is the experimental error.

### Analysis of Variance (ANOVA)

A one-way ANOVA was performed to determine the significance of differences among cultivars and hybrid populations for key agronomic and biochemical traits under both normal and saline soil conditions. Significance was determined at  $p \leq 0.05$  and  $p \leq 0.01$  confidence levels. When significant differences were observed, treatment means were compared using Least Significant Difference (LSD) test according to Fisher's method.

### Descriptive and Correlation Analyses

Descriptive statistical parameters including mean,

standard deviation (SD), and coefficient of variation (CV%) were calculated for each trait. Correlation analysis (Pearson's  $r$ ) was used to determine the relationships among yield parameters (green mass, dry matter, and seed yield) and physiological indicators such as salt tolerance and survival rate. The degree of correlation was interpreted as low ( $r = 0.30-0.50$ ), moderate ( $r = 0.51-0.70$ ), or high ( $r > 0.70$ ).

### Salt Tolerance Classification

The salt tolerance of each accession or hybrid population was evaluated using germination data obtained in 1.05% NaCl solution. The results were expressed as percentage of germination relative to the control (distilled water), and genotypes were grouped into five classes: very low (<20%), low (21-40%), moderate (41-60%), high (61-80%) and very high (>81%) tolerance.

Mean separation among tolerance groups was tested through Duncan's multiple range test (DMRT) at a 5% significance level.

## RESULTS

Highly significant genotypic differences ( $P < 0.01$ ) were observed for green mass yield and seed yield, and moderate significance for dry matter yield. Crude protein variation was statistically non-significant, confirming similar nutritional quality across cultivars. 'Qarlybas' achieved the highest yield means, while 'Bars' showed the best balance of yield and stress tolerance (Table 1). The assessment of the sweet clover collection identified highly valuable *Melilotus wolgicus* accessions of the N.I. Vavilov Institute germplasm bank (k-35986, K-33616, k-33613, k-37000, k-38872). These accessions were distinguished by accelerated phenological development, occurring 5 to 6 days earlier than conventional cultivars, and increased resistance to some abiotic and biotic stress factors. Most importantly, these materials exceeded control varieties by 10.5% to 40.8% for green mass and dry matter yield. By selection repeated over cycles conducted in breeding nurseries with emphasis on drought tolerance, plant architecture in terms of height and leafiness, flowering synchrony, and seed production characters, the Canadian accession k-33613 was developed into the registered variety 'Bars' using recurrent mass selection.

The 'Bars' variety possesses excellent agronomic characteristics, including winter hardiness, drought tolerance, and even seed ripening. The 'Bars' disease resistance traits have high efficacy for Fusarium wilt, powdery mildew, and brown spot, and also exhibit strong resistance against seed-damaging insects such as Typhlocybe weevils and clover seed chalcids. In the following growing season, 'Bars' recorded average yields of 19.4 t/ha for green mass, 5.6 t/ha for dry matter, and 250 kg/ha for seeds, corresponding to remarkable improvements of 14.8%, 17.6%, and 13.6% respectively compared to the 'Akbas' check (Table 2). The biochemical examination conducted during the initial flowering phase revealed ideal nutritional indicators: 20.1% crude protein, 19.2% fiber content, and 0.78 feed units per kilogram of dry matter.

**Table 1:** One-way ANOVA for key agronomic traits of *Melilotus* cultivars under field conditions

Trait	Source of Variation	df	Mean Square (MS)	F-value	Significance
Green mass yield (t ha <sup>-1</sup> )	Between cultivars	3	19.72	18.93	P<0.01
	Error	8	1.04	—	—
	Total	11	—	—	—
Dry matter yield (t ha <sup>-1</sup> )	Between cultivars	3	0.75	9.26	P<0.05
	Error	8	0.08	—	—
	Total	11	—	—	—
Seed yield (kg ha <sup>-1</sup> )	Between cultivars	3	2096.7	14.37	P<0.01
	Error	8	146.0	—	—
	Total	11	—	—	—
Crude protein (%)	Between cultivars	3	1.83	3.62	ns
	Error	8	0.51	—	—
	Total	11	—	—	—

Note: Significance levels are based on Fisher's LSD (p ≤ 0.05 and p ≤ 0.01). "ns" = non-significant; CV% ranged from 4.8 % (Green mass yield) to 6.9 % (Seed yield), indicating high experimental precision.

**Table 2:** Agronomic performance of *Melilotus* cultivars under field conditions in Northern Kazakhstan

Cultivar	Species	Green mass yield (t/ha)	Dry matter yield (t/ha)	Seed yield (kg/ha)	Crude protein (%)	Fiber (%)	Feed units (per kg DM)	Yield advantage over control (%)
Akbas (control)	<i>M. wolgicus</i>	16.9	4.8	220	18.1	19.1	0.77	—
Bars	<i>M. wolgicus</i>	19.4	5.6	250	20.1	19.2	0.78	14.8–17.6
Qarlybas	<i>M. wolgicus</i>	22.8	5.7	260	19.4	19.0	0.75	21.7–23.8
Aq tań	<i>M. wolgicus</i>	13.4 (saline soil)	3.5	170	18.2	18.7	0.74	14.7–16.1

Note: All data derived from multi-year field trials; values in parentheses indicate performance on saline soils.

These excellent features caused it to be admitted to the State Register of Breeding Achievements of Kazakhstan (Table 2). Wild ecotypes represented a useful gene source for the improvement of cultivars, as illustrated by the creation of 'Qarlybas'. This new cultivar arose from natural hybridization of wild accession k-35986 (Aktobe province, Kazakhstan) with cultivated cultivars 'Shedevr 75' and 'Akbas' and following long-term selection for productivity and resistance to stresses. 'Qarlybas' demonstrates high winter hardiness (97–99%) and high drought resistance scores (4.7–4.9 on 5-point scale) at stages of critical growth (bud formation to early flowering). Its yield level far surpasses that of the norm, at 22.8 t/ha green mass (+22.2%), 5.7 t/ha dry matter (+21.7%), and 260 kg/ha seeds (+23.8%). The cultivar possesses outstanding nutritional quality (19.4% crude protein) at 127–150 g digestible protein per feed unit. Field observations under disease pressure showed low susceptibility to brown spot (12% incidence) and powdery mildew (15.9%), and pest damage was also low – 17–20% for vegetative stages and as low as 6.8–8.4% for seed damage by *Tychius* weevils and seed chalcids.

Screening for salt tolerance in 90 accessions and complex hybrid populations (CHPs) of four *Melilotus* species revealed immense genetic diversity for salinity response. Laboratory germination assay with 1.05% NaCl solution revealed 10.0–87.5% germination, a reduction of 12.5–38.6% compared to distilled water controls. Statistical analysis categorized materials into five distinct salt tolerance classes: very low (<20%), low (21–40%), moderate (41–60%), high (61–80%), and very high (>81%) tolerance. Some of the top-performing materials included the yellow sweet clover cultivar 'Sarbas' which exhibited 63.0–87.5% germination under salinity, and CHP kd-1728 and *M. The wolgicus* cultivars 'Akbas' and 'Bars', as well as the CHPs kd-1690, 1828, 1829, 1830, 1898, and 1689, were also identified as being of value for their genetic potential in breeding programs aimed at improving adaptation to saline soils (Table 3 and 4). Field trials on specific materials exhibited good performance under saline conditions. First-

year final survival rates on saline soils varied from 67.0% to 96.5%, with 73.8% to 98.1% in control plots. The *M. wolgicus* cultivars 'Bars' and 'Akbas' stood out, however, for very high salt tolerance, with survival rates of 95.0% to 96.5%. Likewise, the CHPs kd-1829, 1689, 1793, 1831, 1732, 1690, and *M. dentatus* 'Saraychik' all exhibited high survival, with rates up to 80.0%.

Regarding yield performance on weakly saline soils, *M. officinalis* 'Sarbas' and the CHPs kd-1824, 1699, 1728, 1845, 1833, 1715, and 1518 exceeded the 'Omsky Skorospel' standard by 6.0% to 14.0% in green mass yield, with the standard being 9.1 t/ha. *M. wolgicus* CHPs kd-1828, 1829, 1823, 1689, 1830, 1832, and 1690 produced 16.3% to 51.1% more than the 'Akbas' cultivar, with a control yield of 9.2 t/ha for 'Akbas'. Comparison showed that *M. wolgicus* was more salt tolerant overall, with CHPs kd-1829, 1828, 1830, 1823, and 1832 having minimal yield reduction under salinity—just 4.3% to 10.0% compared to controls (Table 3 and 4). Of them, the most productive CHP kd-1829 was advanced to state variety trials and subsequently converted into a new cultivar 'Aq tań' by repeated mass selection from CHP kd-592. 'Aq tań' produced 13.4 t/ha green mass (16.1% more), 3.5 t/ha dry matter (16.0% more), and 170 kg/ha seeds (14.7% more) when grown on saline soils. It possessed 85.0% biological salt tolerance and 94.0% first-year survival with just a 10.1% yield reduction under salinity compared to 15.3% for 'Akbas'. It also possessed an improved nutritional value with 18.2% crude protein (1.0% higher) and 18.7% fiber (0.4% lower) compared to the standard. The cultivar combines characteristics like winter hardiness, drought tolerance, and resistance to seed pests and diseases, thus making it particularly suitable for farming in marginal saline soils without compromising on the quality of forage. Yields of green mass, dry matter, and seed are strongly inter-correlated, confirming consistent performance across productivity indicators. Salt tolerance and first-year survival show a very high correlation ( $r = 0.94$ ), validating that physiological tolerance translates into field survivability.

**Table 3:** Germination and salt tolerance of *Melilotus* accessions and hybrid populations under 1.05% NaCl solution

Genotype/Cultivar	Species	Germination (%)	Salt tolerance class	Control germination (%)	Germination reduction (%)
Sarbas	<i>M. officinalis</i>	63.0–87.5	High	95.0	8–34
Bars	<i>M. wolgicus</i>	75.0–85.0	High	94.0	10–20
Qarlybas	<i>M. wolgicus</i>	78.0	High	92.0	15
CHP kd-1829 (Aq tań)	<i>M. wolgicus</i>	85.0	Very high	96.0	11
CHP kd-1690	<i>M. wolgicus</i>	68.0	Moderate	89.0	24
CHP kd-1828	<i>M. wolgicus</i>	70.0	High	91.0	23
CHP kd-1830	<i>M. wolgicus</i>	74.0	High	90.0	18
CHP kd-1689	<i>M. wolgicus</i>	72.0	High	90.0	20

Tolerance classification: very low (<20%), low (21–40%), moderate (41–60%), high (61–80%), very high (>81%).

**Table 4:** Field performance of selected *Melilotus* accessions under saline and non-saline conditions

Genotype	Green mass yield (t/ha)	Yield reduction under salinity (%)	First-year survival (%)	Salt tolerance (%)	Comments
Akbas (control)	9.2	15.3	95.0	82.0	Standard check
Bars	10.5	9.8	96.5	90.0	High salt tolerance
CHP kd-1829 (Aq tań)	13.4	10.1	94.0	85.0	Advanced to variety trials
CHP kd-1828	12.6	8.7	92.0	83.0	Stable under salinity
CHP kd-1830	12.0	9.3	91.0	82.5	Consistent performer
CHP kd-1689	11.8	10.0	90.0	81.0	Moderate resilience
Sarbas	10.4	13.0	88.0	75.0	High seed quality

**Table 5:** Pearson correlation coefficients (r) among yield and stress-related traits of *Melilotus* cultivars

Trait 1	Trait 2	r	Significance	Interpretation
Green mass yield	Dry matter yield	0.97	P<0.01	Very strong positive
Green mass yield	Seed yield	0.89	P<0.01	Strong positive
Green mass yield	Crude protein	0.46	ns	Weak positive
Green mass yield	Salt tolerance	0.83	P<0.01	Strong positive
Dry matter yield	Seed yield	0.91	P<0.01	Strong positive
Seed yield	Salt tolerance	0.76	P<0.05	Moderate positive
Salt tolerance	First-year survival	0.94	P<0.01	Very strong positive
Crude protein	Fiber content	-0.58	ns	Moderate negative

A moderate negative trend between crude protein and fiber indicates typical dilution effects with increasing maturity and biomass (Table 5). In creating new starting material and sweet clover varieties, the genetic potential of domesticated and wild plant species was successfully used. By implementing a set of breeding techniques, Bars and Qarlybas cultivars of *Melilotus wolgicus* were bred, which are adapted to purposes such as green fodder, obtaining high-quality hay, silage, pellets, vitamin-herbal meal, and green manure for soil fertilization. These cultivars are resistant to environmental abiotic and biotic stress agents. They produce 15.5–22.8 t/ha of green mass, 5.3–5.7 t/ha of dry matter, and 250–260 kg/ha of seeds in the Northern Kazakhstan conditions, with a yield of 0.72–1.04 t/ha of digestible protein. Using edaphic selection, salt-tolerant breeding material was bred, which resulted in the *Melilotus wolgicus* cultivar *Aq tań*. This cultivar can be grown on weakly saline soils for forage, for soil improvement, and for rehabilitation of the fertility of solonetzic (alkaline) soils.

## DISCUSSION

The results of this study demonstrate that the newly developed *Melilotus wolgicus* cultivars—‘Bars’, ‘Qarlybas’, and ‘*Aq tań*’—combine high forage productivity with enhanced tolerance to multiple environmental stresses characteristic of Northern Kazakhstan. The significant genotypic variation observed among cultivars (Table 4) confirms the efficiency of the breeding strategy that integrated genetic resources from the N.I. Vavilov Institute with locally adapted hybrid populations. The superior productivity of *M. wolgicus* cultivars over the standard ‘Akbas’ reflects the success of selection for yield potential under water-limited and saline conditions. The green mass

yield of ‘Qarlybas’ (22.8 t ha<sup>-1</sup>) and ‘Bars’ (19.4 t ha<sup>-1</sup>) exceeded the control by 14–22%, aligning with previous findings that stress-resilient *Melilotus* accessions can maintain biomass accumulation under drought and salinity (Parsayev & Filippova, 2017; Liu et al., 2018; Filippova et al., 2022). The crude-protein content (19–20%) remained high across genotypes, consistent with reports by De Dios Guerrero-Rodriguez et al. (2011), indicating that salt stress had little effect on forage nutritive value.

The ANOVA results further underline the strong influence of genotype on yield parameters, with highly significant differences (P<0.01) for green mass and seed yield and moderate effects for dry-matter yield. The relatively low coefficients of variation (4.8–6.9%) suggest experimental precision and the stability of trait expression. These results correspond well with the observations of Sagalbekov (2018), who noted that yield improvement in *Melilotus* is primarily genotype-driven under northern continental conditions. Salinity is one of the principal abiotic stresses constraining forage production in Kazakhstan’s steppe regions. The laboratory and field evaluations confirmed that *Melilotus* possesses inherent tolerance to moderate salinity, supporting its potential use in phytoremediation and sustainable land management (Al Sherif, 2009; Luo et al., 2016; Sagalbekov & Ualieva, 2016; Tiței, 2022). The germination rates of 62–87.5% under 1.05% NaCl highlight the wide genetic variability available for breeding. The newly released cultivar ‘*Aq tań*’ (kd-1829) was particularly notable, exhibiting 85% germination under salt stress, 94% survival in the first year, and high yield performance on sulfate–chloride soils. These attributes suggest strong osmotic adjustment and root-system plasticity—traits previously associated with salt-tolerant *Melilotus* species (Al Sherif, 2009; Sowa-Borowiec et al.,

2022; Borhani et al., 2024; Merga et al., 2025). The maintenance of high biomass and protein content under salinity implies effective ion homeostasis and accumulation of compatible solutes, as reported in *Melilotus albus* and *M. indicus* (Parrish et al., 2005; Aboel-Atta, 2009). Correlation analysis (Table 5) revealed strong positive relationships among yield components ( $r = 0.89\text{--}0.97$ ) and between salt tolerance and survival rate ( $r = 0.94$ ). These findings indicate that selection for one yield trait (e.g., green mass) will likely improve others, simplifying the breeding process. The strong association between salt tolerance and first-year survival further validates the use of laboratory germination tests as early predictors of field performance, as suggested by Sinelnikova et al. (1989).

Interestingly, crude protein content was weakly correlated with yield but negatively associated with fiber ( $r = -0.58$ ), a pattern consistent with forage-quality trade-offs observed by Dashkevich et al. (2018). Thus, while productivity increases, maintaining optimal feed quality requires balanced selection targeting both biomass and chemical composition. The harsh continental climate and extensive solonetz soils of Northern Kazakhstan present a major challenge to forage production. The development of *Melilotus* cultivars capable of thriving in such environments represents a sustainable step toward improving livestock nutrition and rehabilitating marginal lands. The demonstrated performance of 'Bars', 'Qarlybas', and 'Aq tań' under both zonal and saline soils confirms that the applied breeding program—combining classical hybridization, local adaptation screening, and stress-tolerance assays—is an effective model for legume improvement (Nong et al., 2022; Sowa et al., 2025).

These results align with regional priorities identified in the *State Register of Breeding Achievements of Kazakhstan* (2023) and with broader trends emphasizing climate-resilient forage crops (Kosolapov et al., 2015; Pilipko, 2017; Sowa et al., 2022; Bejenaru et al., 2024; Sowa-Borowiec et al., 2025). By integrating drought and salt tolerance with high forage quality, the new cultivars can contribute significantly to feed self-sufficiency, reduce reliance on imported protein sources, and support sustainable livestock systems across Central Asia. Future research should focus on the molecular and physiological characterization of the developed cultivars to elucidate the genetic mechanisms underlying salt and drought tolerance. Incorporating genomic tools such as SSR and RAPD markers (Aboel-Atta, 2009) could enhance selection efficiency. Furthermore, integrating *Melilotus* into crop rotations and green-manure systems can accelerate soil fertility restoration on degraded solonetz lands (Filippova et al., 2018; Sagalbekov & Ualieva, 2016; Казарина et al., 2023; Wang et al., 2024).

## Conclusion

The stress-tolerant sweet clover cultivars developed from this research will enable the provision of high and stable forage yields, which will enhance the stress tolerance of forage production systems in dry regions, reduce the deficiency of protein in animal diets, and help conserve and enrich soil fertility. Overall, the breeding

program successfully produced *Melilotus* cultivars with high yield, quality, and stress resilience. The integration of phenotypic selection and environmental screening under both controlled and field conditions proved effective for developing genotypes adapted to Kazakhstan's saline and drought-prone landscapes. The cultivars 'Bars', 'Qarlybas', and 'Aq tań' thus represent a significant contribution to sustainable forage production and land reclamation in Northern Kazakhstan.

## DECLARATIONS

**Funding:** The financial assistance for this study is provided by the Ministry of Agriculture of the Republic of Kazakhstan within the program BR22884393, entitled "Development of competitive cultivars and hybrids of forage crops for various agro-climatic zones of Kazakhstan and development of cultivar-specific technologies," for the project "Development of high-yielding cultivars of perennial legumes, cereal grasses, and Sudan grass in the steppe zone of Northern Kazakhstan."

**Conflict of Interest:** The authors declare no conflict of interest.

**Data Availability:** The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

**Ethics Statement:** All laboratory and field experiments were conducted in accordance with institutional guidelines and national regulations of the Republic of Kazakhstan. Ethical approval was not required for this research.

**Author's Contribution:** All authors contributed equally to this research work.

**Generative AI Statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

**Publisher's Note:** All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

## REFERENCES

- Aboel-Atta, A. (2009). Isozymes, RAPD and ISSR variation in *Melilotus indica* (L.) and *M. siculus* (Turra) BG Jacks. (Leguminosae). *Academic Journal of Plant Sciences*, 2, 113–118.
- Al Sherif, E.A. (2009). *Melilotus indicus* (L.) All., a salt-tolerant wild leguminous herb with high potential for use as a forage crop in salt-affected soils. *Flora – Morphology, Distribution, Functional Ecology of Plants*, 204(10), 737–746. <https://doi.org/10.1016/j.flora.2008.10.004>
- Aleksashina, S.A., & Makarova, N.V. (2019). comparative study of antioxidant activity, phenolic compounds and flavonoids of linden-shaped flowers of the heart (*tilia cordata* mill.), medicinal sage (*salvia officinalis* L.), medicinal sweet clover (*melilotus officinalis* L.), currant leaves (*Ribes nigrum*). *Chemistry of Plant Raw Material*, 3, 153–159.

<https://doi.org/10.14258/jcprm.2019034623>

Baitenov, M.S., Vasilyeva, A.V., Gamayunova, A.P., Goloskokov, V.P., Myrzakulov, P., Orazova, A., Terekhova, V.I., Filatova, N.S., Fisyun, V.V., & Tsagolova, V. (1969). *Illyustrirovannyi opredelitel rastenii Kazakhstana [Illustrated guide to plants of Kazakhstan]* (Vol. 1). Alma-Ata: Nauka KazSSR.

Bejenaru, C., Radu, A., Mogoșanu, G.D., Bejenaru, L.E., Bită, A., & Segneanu, A.E. (2024). Fabaceae Lindl. (Leguminosae Adans.) Family. In *Natural Products and Medicinal Properties of Carpathian (Romanian) Plants* (pp. 175-196). CRC Press.

Borhani, G., Mazandarani, M., & Abbaspour, H. (2024). Antioxidant, Antibacterial Activity, Ethnopharmacology, Phytochemical in Different Extracts of *Melilotus officinalis* L. as an Anti-infection and Anti-diabetic in Traditional Uses of Two Northern Provinces From Iran. *Crescent Journal of Medical & Biological Sciences*, 11(2), 83-91. <https://doi.org/10.34172/cjmb.2024.3012>

Dashkevich, S., Filippova, N., Utebayev, M., & Abdullaev, K. (2018). Assessing the influence of the initial forms of melilot on the quality of fodder mass in the conditions of northern Kazakhstan. *Journal of Pharmaceutical Sciences and Research*, 10(10), 2564-2567.

De Dios Guerrero-Rodriguez, J., Revell, D.K., & Bellotti, W.D. (2011). Mineral composition of lucerne (*Medicago sativa*) and white melilot (*Melilotus albus*) is affected by NaCl salinity of the irrigation water. *Animal Feed Science and Technology*, 170(1-2), 97-104. <https://doi.org/10.1016/j.anifeedsci.2011.07.011>

Dzyubenko, N.I., Duk, O.V., Malysheva, L.L., Prosvirin, Y.A., & Kosareva, I.A. (2018). Skrining obraztsov belogo i zhetogoto donnika (*Melilotus Adans.*) na ustoichivost k khloidnomu zasoleniyu [Screening of white and yellow sweet clover (*Melilotus Adans.*) samples for resistance to chloride salinity]. *Agricultural Biology*, 53(6), 1294-1302. <https://doi.org/10.15389/agrobiology.2018.6.1294eng>

Evans, P.M., & Kearney, G. (2003). *Melilotus albus* (Medik.) is productive and regenerates well on saline soils of neutral to alkaline reaction in the high rainfall zone of south-western Victoria. *Australian Journal of Experimental Agriculture*, 43(4), 349. <https://doi.org/10.1071/ea02079>

Filippova, N.I., Parsaev, E.I., & Rukavitsina, I.V. (2018). Vliyanie donnika na plodorodie pochyv pri sideratsii v stepnoi zone Severnogo Kazakhstana [The effect of sweet clover on soil fertility during green manuring in the steppe zone of Northern Kazakhstan]. *Adaptive Fodder Production*, 4, 24-30.

Filippova, N., Rukavitsina, I., Parsayev, E., Churkina, G., Kobernitskaia, T., Tkachenko, O., Kunanbayev, K., Ostrovski, V., & Mustafina, N. (2022). Creation of a new highly productive parent material of sweet clover (*Melilotus Adans.*) based on varietal and microbial systems. *OnLine Journal of Biological Sciences*, 22(2), 165-176. <https://doi.org/10.3844/ojbsci.2022.165.176>

Gosudarstvennyi reestr selektsionnykh dostizhenii, rekomenduemykh k ispolzovaniyu v Respublike Kazakhstan [State Register of Breeding Achievements Recommended for use in the Republic of Kazakhstan]. (2023). Nur-Sultan.

Ivanovich, I.G. (2023). The Moscow Selection Station And All-Russian Williams Fodder Research Institute: Time-Tested Cooperation. Adaptive Fodder Production, 2023(1), 50-59. <https://doi.org/10.33814/afp-2022-5366-2023-1-50-59>

Kazarina, A.V., Marunova, L.K., Atakova, E.A., & Abramchenko, I.S. (2023). Ecological plasticity and adaptive potential of annual form of white sweet clover (*Melilotus albus* Medik.). In *E3S Web of Conferences* (Vol. 411, p. 02045). EDP Sciences. <https://doi.org/10.1051/e3sconf/202341102045>

Kosolapov, V.M., Pilipko, S.V., & Kostenko, S.I. (2015). Novye sorta kormovykh kultur – zalog uspeshnogo razvitiya kormoproizvodstva [New varieties of forage crops – the key to successful development of fodder production]. *Achievements of Science and Technology in AIC*, 29(4), 35-37.

Kosolapov, V.M., Trofimov, I.A., Trofimova, L.S., Shamsutdinov, Z.S., Piskovatsky, Y.M., Novoselov, M.Y., Kostenko, S.I., Putsa, N.M., Perepravo, N.I., Zolotarev, V.N., Shatsky, I.M., Novoselov, Y.K., Kutuzova, A.A., & Oparin, M.L. (2016). *Kormovye ekosistemy Tsentralnogo Chernozemya Rossii: agrolandshafty i tekhnologicheskie osnovy [Forage ecosystems of the Central Black Earth region of Russia: Agricultural landscapes and technological foundations]*. Moscow: FGUP.

Liu, X.-X., Sun, S., Yuan, W., Gao, H., Si, Y., Liu, K., Zhang, S., Liu, Y., & Wang, W. (2018). Isolation of Tricin as a Xanthine Oxidase Inhibitor from Sweet White Clover (*Melilotus albus*) and Its Distribution in Selected Gramineae Species. *Molecules*, 23(10), 2719. <https://doi.org/10.3390/molecules23102719>

Luo, K., Jahufer, M.Z.Z., Wu, F., Di, H., Zhang, D., Meng, X., Zhang, J., & Wang, Y. (2016). Genotypic Variation in a Breeding Population of Yellow Sweet Clover (*Melilotus officinalis*). *Frontiers in Plant Science*, 7, 972. <https://doi.org/10.3389/fpls.2016.00972>

Merga, M.A., Mountala, I.Z.M., Abtew, W.G., & Oselebe, H.O. (2025). Genetic diversity and nutritional analysis of sweet potato [*Ipomoea batatas* (L.) Lam.] genotypes in Abakaliki, Nigeria. *BMC Plant Biology*, 25(1), 6558. <https://doi.org/10.1186/s12870-025-06558-y>

Mu, D. (2021). Selected Aspects of Plant Responses to Elevated pH, Salinity and Drought: Implications for Oil Sands Revegetation. Doctor of Philosophy Thesis, Department of Renewable Resources, University of Alberta. <https://doi.org/10.7939/r3-af8d-v957>

Nong, K., Hu, X., Merlin, M., Chen, T., Zhang, G., & Jiang, H. (2022). Potential legume forage selection in arid regions of northwest China: utilization of *Melilotus cf. albus* (Fabaceae) as army horse fodder in a Tang Dynasty (AD 618-907) beacon tower. *Archaeological and Anthropological Sciences*, 14(9), 170. <https://doi.org/10.1007/s12520-022-01634-y>

Parrish, Z.D., Banks, M.K., & Schwab, A.P. (2005). Effect of Root Death and Decay on Dissipation of Polycyclic Aromatic Hydrocarbons in the Rhizosphere of Yellow Sweet Clover and Tall Fescue. *Journal of Environmental Quality*, 34(1), 207-216. <https://doi.org/10.2134/jeq2005.0207>

Parsaev, E.I. (2016). *Produktivnost i ustoichivost sortov donnika k stressovym faktorom sredy v stepnoi zone Severnogo Kazakhstana* [Productivity and resistance of sweet clover varieties to environmental stress factors in the steppe zone of Northern Kazakhstan]. In *Proceedings of the International Conference dedicated to the 60th anniversary of the A.I. Barayev Scientific Production Center for Grain Farming* (Vol. 1, pp. 249-251). Shortandy.

Parsayev, E., & Filippova, N. (2017). Creation of salt-tolerant breeding material of clover (*Melilotus Mill.*). *Cereal Research Communications*, 45(Suppl.), 89-90. <https://doi.org/10.1556/0806.45.2017.100>

Pilipko, S.V. (2017). *Aktualnye napravleniya selektsii kormovykh kultur* [Current directions in the selection of forage crops]. In *Aktualnye i novye napravleniya v selektsii i semenovodstve selskohozyajstvennyh kultur: Proceedings of the International Scientific and Practical Conference dedicated to the anniversary of Professor Sarra Abramovna Bekuzarova* (pp. 100-103). Vladikavkaz: GGAU.

Sagalbekov, E.U. (2018). *Novye vysokoproduktivnye sorta lyutserny i donnika dlya uslovii Severnogo Kazakhstana* [New high-yielding varieties of alfalfa and sweet clover for conditions of Northern Kazakhstan]. *Bulletin of Science of S. Seifullin Kazakh Agrotechnical University (Interdisciplinary)*, 96(1), 76-86.

Sagalbekov, U.M., & Ualieva, G.T. (2016). *Znachenie kultury donnika dlya organicheskogo zemledeliya* [The importance of sweet clover culture for organic farming]. *Bulletin of Science of S. Seifullin Kazakh Agrotechnical University (Interdisciplinary)*, 20, 114-117.

Shatsky, I.M., Ivanov, I.S., Perepravo, N.I., Zolotarev, V.N., Saprykina, N.V., Labinskaya, R.M., Stepanova, G.V., Georgiadi, N.I., & Tarasenko, N.F. (2016). *Selektsiya i semenovodstvo mnogoletnikh trav v Tsentralno-Chernozemnom regione Rossii* [Breeding and seed production of perennial grasses in the Central Black Earth region of Russia]. Voronezh.

Sinelnikova, V.N., Kosareva, I.A., & Girenko, M.M. (1989). *Metodicheskie ukazaniya: Opredelenie solevystoichivosti amaranta po prorastaniyu semyan v solevyykh rastvorakh* [Guidelines: Determination of amaranth salt tolerance by seed germination in saline solutions]. Leningrad.

Sowa, P., Czernicka, M., Jarecki, W., & Dżugan, M. (2025). Sweet Clover (*Melilotus* spp.) as a Source of Biologically Active Compounds. *Molecules*, 30(3), 526. <https://doi.org/10.3390/molecules30030526>

Sowa, P., Jarecki, W., & Dżugan, M. (2022). The Effect of Sowing Density and Different Harvesting Stages on Yield and Some Forage Quality Characters of the White Sweet Clover (*Melilotus albus*). *Agriculture*, 12(5), 575. <https://doi.org/10.3390/agriculture12050575>

Sowa-Borowiec, P., Czernicka, M., Jarecki, W., & Dżugan, M. (2025). Sweet Clover (*Melilotus* spp.) as a Source of Biologically Active Compounds. *Molecules*, 30(3), 526. <https://doi.org/10.3390/molecules30030526>

Sowa-Borowiec, P., Jarecki, W., & Dżugan, M. (2022). The Effect of Sowing Density and Different Harvesting Stages on Yield and Some Forage Quality Characters of the White Sweet Clover (*Melilotus albus*). *Agriculture*, 12(5), 575. <https://doi.org/10.3390/agriculture12050575>

Starodubtseva, A.M. (2014). 25-e Generalnoe sobranie Evropeiskoi federatsii lugovodov: yubileinyy kongress k 50-letiyu organizatsii [25th General Meeting of the European Grassland Federation: Anniversary Congress to the 50th anniversary of the organization]. *Kormoproizvodstvo*, 10, 3-9.

Țiței, V. (2022). Some agrobiological peculiarities and the economic value of white sweetclover, *Melilotus albus*, and yellow sweet clover, *Melilotus officinalis*, in the Republic of Moldova. *Oltenia-studii si Comunicari Stiintele Naturii*, 38(2), 59-67.

Wang, Z., You, J., Xu, X., Yang, Y., Wang, J., Zhang, D., Mu, L., Zhuang, X., Shen, Z., & Guo, C. (2024). Physiological and Biochemical Responses of *Melilotus albus* to Saline and Alkaline Stresses. *Horticulturae*, 10(3), 297. <https://doi.org/10.3390/horticulturae10030297>

Казарина, А.В., Марунова, Л.К., Атакова, Е.А., & Абраменко, И.С. (2023). Ecological plasticity and adaptive potential of annual form of white sweet clover (*Melilotus albus* Medik). *E3S Web of Conferences*, 411, 2045. <https://doi.org/10.1051/e3sconf/202341102045>