AGRICULTURAL BIOLOGY,
2018, V. 53, № 6, pp. 1294-1302
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.366:631.524.85:581.1

doi: 10.15389/agrobiology.2018.6.1294eng
doi: 10.15389/agrobiology.2018.6.1294rus

SCREENING OF SWEET CLOVER (*Melilotus* Adans.) SPECIES DIVERSITY FOR RESISTANCE TO CHLORIDE SALINIZATION

N.I. DZYUBENKO, O.V. DUK, L.L. MALYSHEV, Yu.A. PROSVIRIN, I.A. KOSAREVA

Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, 42-44, ul. Bol'shaya Morskaya, St. Petersburg, 190000 Russia, e-mail irkos2004@yandex.ru (✉ corresponding author)
ORCID:
Dzyubenko N.I. orcid.org/0000-0003-0250-5814
Duk O.V. orcid.org/0000-0001-9441-5802
Malyshev L.L. orcid.org/0000-0002-8595-1336
Prosvirin Yu.A. orcid.org/0000-0001-8018-8240
Kosareva I.A. orcid.org/0000-0003-2522-5575
The authors declare no conflict of interests
Acknowledgements:
The work was performed according to the State Assignment (VIR topic No. 0662-2018-0016)
Received October 15, 2017

Abstract

Saline soils are widespread in the world, including in CIS countries. Species of sweet clover (genus *Melilotus* Adans.) which are valuable fodder plants, e.g. at insufficient moisture on chestnut saline soils, are also among the best phytomeliorants in the legume family. However, during early stages of growing the sweet clover has poor salinity tolerance. Here, we modified earlier developed test and screened accessions of the VIR World Collection (Vavilov All-Russian Institute of Plant Genetic Resources) for resistance to chloride salinity, and for the first time identified among them the forms with high salinity resistance. In the research we used a laboratory method of assessing salt tolerance. Samples of white and yellow sweet clover were grown under controlled conditions, in water culture, according to the following scheme: control (without salinity); salinity of 3 atm. NaCl; the salinity of 5 atm. NaCl. The length of root and sprout of seedlings were measured after 5-day exposure to the stressor. For each sample we calculated the indices of the root length and the sprout length as the ratio of the relevant medium parameters of plants in the test to that in the control. The higher the index value, the more salt-tolerant the sample is. In our tests, chloride salinity caused growth inhibition of roots and shoots samples of both species. The degree of negative growth response intensifies with an increase in NaCl concentration. It was shown that salt stressor intensifies intra- and interpopulation variability of sweet clover growth indicators. It is established that the root length index is more informative diagnostic criterion for laboratory screening for salt tolerance among sweet clover plants. The studied fragment of the collection includes 36.2 % of resistant samples of white sweet clover and 29.6 % of yellow sweet clover, i.e. the adaptive capacity towards excess of sodium chloride in the root zone of the studied species is approximately the same. Salt-resistant varieties of white clover come from Far East (Ryadovoi and Diomid), Kazakhstan (Shedevr 75), Novosibirsk (Lucernovidnyi D-20) and Ivanovo (wild sample k-15650) regions; of yellow sweet clover — from Western Siberia (Omskii 8, Omskii 916, Novosibirskii 1, Severotatarskii, Khorog 2155). Selected salt tolerant forms can serve as initial parental forms for creation of yellow and white sweet clover varieties resistant to chloride salinity during early stages of plant development. This will allow better use of the potential of these species for forage, and for bioremediation goals. According to the obtained data, salt-tolerant sweet clover samples often originate from a region with widespread saline soils that should be considered to determine the strategy of search for forms with edaphic resistance valuable for breeding. Many of the selected salt-tolerant varieties are also characterized by other important breeding traits: high productivity, intensive regrowth after cutting, precocity, biotic and abiotic resistance to viruses, drought, low temperatures, unfavorable overwintering, valuable biochemical properties such as high protein content and low coumarin level.

Keywords: chloride salinity, *Melilotus*, sweet clover, salt tolerance, growth indicators, variability, root length index

Saline soils of different types and salinization degrees occupy about 550000 km^2 of the former USSR territory. In the Russian Federation, they are most widespread in the North Caucasus, in the Lower Volga Region, in the Southern Urals, and in Western Siberia. As an effective method for improving the state of saline soils, one can consider salinity control by means of cultivation of resistant plant species [1, 2]. Leguminous plants are widely used in soil remediation though, according to some data, display a moderate salt tolerance [3]. Meadows formed on saline soils by perennial leguminous grasses promote soil their enrichment with nitrogen, restoration of fertility and recovery to agriculture.

Species of the genus *Melilotus* Adans. (sweet clover) are among the best phytomeliorants in the family *Fabaceae*. Sweet clover species grow in saline soils where the traditional leguminous pants do not survive [2, 4]. The origin of the genus is Eurasia and North Africa; the representatives of the genus are resistant to heat and cold [5, 6]. Twenty-six species of sweet clover are described; the genus is presented by 13 species in the former USSR area [7]. The following species are integrated into agriculture: sweet clover *M. albus* Medik., yellow melilot *M. officinalis* (L.) Pall., Volga sweet-clover *M. wolgicus* Poir., plaster clover *M. suaveolens* Ledeb., dentated melilot *M. dentatus* (Waldst. et Kit.) Pers., hairy melilot *M. hirsutus* Lipsky, and Caspian melilot *M. caspicus* Grun. [8]. Some samples of dentated melilot and Indian melilot are noted for high salt tolerance [9-11]. In the world, melilot sowings occupy 1.2-1.4 million hectares and are mostly concentrated in Canada and the CIS (Western and Eastern Siberia, the Volga Region, Kazakhstan, Yakutia, the Southern Ural Mountains, the North Caucasus, Bashkiria, the Far East). In the CIS countries, these species are widespread almost in all floral zones [12]. Melilot is a perspective fodder, phytomeliorative, medicinal and melliferous crop; the increasing attention is given to it as green manure [13, 14]. The wide application of melilot is due to its high ecological plasticity. Due to the deeply penetrating root system, melilots can grow in a wide range of edaphic conditions; melilot is drought-resistant, winter-hardy, not exacting to soils fertility [15]. The greatest green mass productivity among perennial leguminous crops is most typical for sweet clover and yellow melilot; sweet clover is more valuable as a fodder [15].

Disorder of the activity of nodule bacteria responsible for the fixation of atmospheric nitrogen [16] becomes one of the main causes of growth oppression of leguminous plants in saline soils. Melilot in the conditions of soil salinization is capable to form rather big green mass yield and to enrich the soil with nitrogen, which makes it applicable for the subsequent cultivation of other crops [15]. Now, research on the use of this culture in the saline soils is conducted in the Central [16], Volga [17, 18], Southern [19-22], North Caucasian [23] and Siberian [24] regions.

Melilot, as well as many other crops [25], displays high sensitivity to the raised salts content in the soil. In this connection, search for melilot forms with high salt tolerance in the initial growth of plants (seed germination and sprout growth) is considered relevant for research.

The collection of melilot of VIR (Vavilov All-Russian Institute of Plant Genetic Resources, St.-Petersburg) contains about 1000 samples (species, varieties, selection and wild-growing samples). The collection is studied for a series of valuable traits, i.e. winter hardiness, biochemical composition, morphological variability [26], resistance to sour soils [27, 28]. Now in Russia, it is admitted to use 15 species of white melilot and 8 species of yellow melilot. In laboratory screening for salt tolerance, one often uses sprouts growth indexes as a test parameter [29, 30], depending both on the stress factor intensity (salts content in the roots zone) and on the genotype properties [31, 32]. In the conditions of chloride salinity, ions of Na and Cl coming into cells compete with the major nutritious elements, first of all, with ions of potassium, calcium and magnesium, which leads to toxic and metabolic stresses in plants, their oppression, and sometimes to destruction at early stages of ontogenesis [33-35]. Now mechanisms of acclimatization of plants in the conditions of the raised salt content in soil [37] have been revealed for some leguminous plants (*Medicago truncatula*

Gaertn.); they are studied in details [38].

In this work, using a modified screening methodology, we for the first time found among VIR collection accessions the forms high-resistant to salinization which are perspective for use as a source of salt tolerance in breeding.

The purpose of the studies is revealing a specific diversity of the collection samples of melilot for resistance to chloride salinity at the early stages of ontogenesis.

Techniques. Fifty-eight samples of white melilot and 27 samples of yellow melilot of various ecogeographical origins, including cultivated and wild-growing forms, were studied. Samples were grown under artificial lightening (10 klx, 16 h photoperiod, day temperature +21 ... +22 °C, night temperature +17 ... +18 °C). The seeds stratified with sandpaper (50 pieces per sample) were put in germinator cells with a mesh bottom. Germinators were placed in containers with distilled water for 2 days so that the grid touched the water surface; above they were covered with a polyethylene film. Not germinating seeds were rejected. Then germinators with seeds starting to germinate were transferred into solutions according to the trial schema: control (distilled water); stress background 1 (NaCl solution with osmotic pressure 3 atm.); stress background 2 (NaCl solution with osmotic pressure 5 atm.). After 5 days, the root and sprout length was measured.

For statistical data processing, Statistica 6.0 software (StatSoft, Inc., the USA) and Systat 10.2 software (Systat Software, Inc., the USA) were used. For each sample, the mean value (M), the standard error of the mean (±SEM) and the variation coefficient (Cv) were calculated. The informative value of indicators was estimated by comparison of the intrapopulation variability of the initial traits (the mean variation coefficient within the populations Cv_1) and their interpopulation variability (the variation coefficient among the means of the populations Cv_2). According to the mean values of the root and sprout length, the root and sprout length indexes (IR and IS) were calculated as a proportion of the index in a trial variant to the control (the higher the index value, the more the sample is resistant to salinization). For splitting plants into groups according to salinity resistance for the totality of the calculated indexes, confidence intervals of the means were calculated. Samples having the index value below the low level of the confidence limits were considered low-tolerant, those from the low and the upper levels of confidence limits were moderately tolerant, and those above the upper level of confidence limits were deemed tolerant.

Results. There are various laboratory methods of plant testing for salt tolerance at the early stages of ontogenesis [39]. To choose an optimum method, we have considered available data on the use of morphometric indexes in diagnostics, in particular, the lengths of sprouts and the roots of seedlings [40-42]. The method to estimate melilot salt tolerance developed earlier [43] assumes the registration of seed germination after salt exposure, that is, it covers earlier stages of plants development, from seed swelling to root emergence. In our opinion, screening indexes, in this case, can depend on seeds quality. According to the developed modification, the diagnostics are carried out for photosynthesizing sprouts, with prior rejection of poor seeds. Besides, such modification provides high performance and reproducibility of the trial.

In the aqueous culture, there was a significant genotypic variability of growth indexes in all variants of the trial. In control (absence of salinization), the most intensive growth of roots was typical for the varieties of sweet clover Kinelskii (Samara Region), Neosypayushchiisya (Krasnodar Territory), Bashkirskii (Republic of Bashkortostan), Yantar (Irkutsk Region), Mestnyi (Austria). Yellow melilot demonstrated the intensive growth of roots in the absence of salt stressor in the varieties from Canada (Goldtop and Yukon). Wild-growing samples of sweet clover from Kazakhstan (k-48543), the Republic of Yakutia (k-46992), the varieties of yellow melilot Yukon and Norgold (Canada), No. 28, No. 29 (the USA) showed high rate of sprout length growth under the control conditions.

The chloride salinity in the roots zone, as a rule, led to the intensifying intrapopulation and interpopulation variability of growth indexes (with the exception of the interpopulation variability of sprout length in yellow melilot) (Table 1). Both in control and salinization groups, the intrapopulation variability of root length and sprout length mostly exceeded the interpopulation one. Note, in both species in all trial variants, intrapopulation variability of root length was higher compare to sprout length.

Under salinization, the intrapopulation variability of root length increased more significantly than sprout length; therefore, for salt tolerance diagnostics, the length of roots was used. A similar result was obtained in seeking for a diagnostic criterion for the melilot estimation for resistance to aluminum toxicity in sour soils [27, 28].

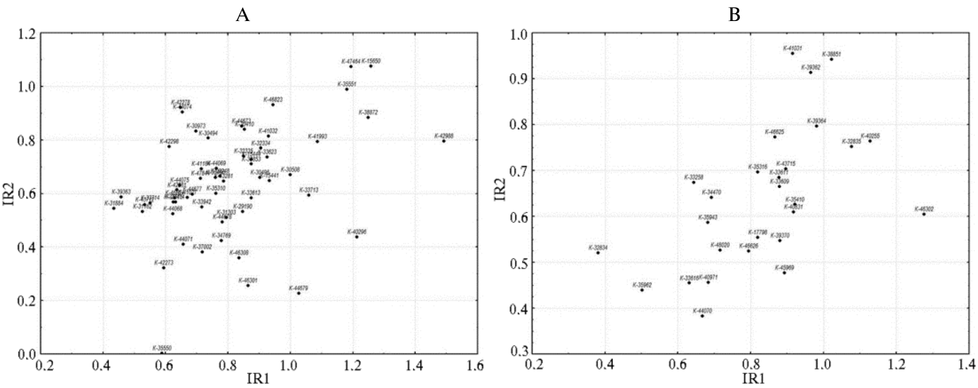
1. Intrapopulation and interpopulation variability of root and sprout length in species of sweet clover *Melilotus albus* Medik. and yellow melilot *M. officinalis* (L.) Pall. depending on cultivation conditions

Indicator	*Melilotus*											
	albus			*officinalis*			*albus*			*officinalis*		
	root length						sprout length					
Osmotic pressure of background, atm.	0	3	5	0	3	5	0	3	5	0	3	5
Intrapopulation variability (Cv_1, %)	45	49	46	34	47	41	24	26	28	23	25	26
Interpopulation variability (Cv_2, %)	21	26	34	22	29	30	19	22	25	26	26	21

In salinization, the repression of sprout growth increased with NaCl concentration increasing. The root length index in sweet clover was 0.44-1.49 (Cv = 3.5 %) at osmotic pressure of 3 atm. and 0.003-1.07 (Cv = 4.2 %) at 5 atm.; for yellow melilot, these values were 0.38-1.28 (Cv = 4.4 %) and 0.38-0.95 (Cv = 4.6 %), respectively.

For allocation of the samples in groups according to their salt tolerance, we used the confidence limits of the mean of the roots length index. The samples were distributed in three conditional groups: tolerant, moderately tolerant and low-tolerant. According to the screening, 36.2 % samples were tolerant to chloride salinity among sweet clover samples and 29.6 % samples among yellow melilot. The greatest part of white melilot (39.7 %) and yellow melilot (37.1 %) samples were low-tolerant. Sweet clover samples that showed a higher salt tolerance originated from the Primorye territory (Ryadovoi and Diomid), Kazakhstan (Shedevr 75) and the Novosibirsk Region (Lutsernovidnyi D-20), that is, from regions with saline soils. High salt tolerance was characteristic of the wild-growing sample k-15650 from the Ivanovo region (Table 2, Fig., available http://www.agrobiology.ru). The root length index of this sample at the maximum NaCl concentration (as well as of the variety Diomid) was the highest. Among the samples of yellow melilot, the greatest salt tolerance was in varieties from Western Siberia Omskii 916, Novosibirskii 1, Severotatarskii, Khorogskii 2155, Omskii 8 (the originators are the Siberian Research Institute of Forages and the Siberian Research Institute of Agriculture) (see Table 2, Fig.). They can be involved in breeding for adaptability to saline soils.

Some wild-growing samples and varieties with high salt tolerance at early ontogenesis also have other valuable traits. So, the variety Shedevr 75 (Aktyubinsk experimental station) is characterized by precocity, fast regrowth after mowing, high drought and winter hardiness, resistance to virus diseases, a high content of protein and a low content of coumarin. The variety Ryadovoi from the Primorye Territory is characterized by the intensity of regrowth after mowing, high winter hardiness, less hard seeds, belongs to low coumarin varieties. The highly productive variety Diomid (Primorskiy Research Institute of Agriculture and VIR Far East Experimental Station) obtained from wild-growing samples by mass selection shows fast regrowth in spring, high fodder properties; it is resistant to drought and unfavorable factors of overwintering, rather resistant to fungal diseases. The variety Omsk 8 shows intensive regrowth after mowing, high productivity, drought resistance, winter hardiness and a high content of protein. Important traits for selection, such as high productivity, intensive regrowth after mowing, precocity, bio- and abiotic resistance (to viruses, drought, low temperatures, unfavorable factors of overwintering), and valuable biochemical properties (high content of protein, low coumarin) are typical for many of the salinity-resistant varieties studied (the data are not shown).



Allocation of samples of sweet clover *Melilotus albus* Medik. (A) **and yellow melilot *Melilotus officinalis* (L.) Pall.** (B) **according to salt tolerance at two salinization environments:** IR1 — the root length index at osmotic pressure of 3 atm., IR2 — the root length index osmotic pressure of 5 atm.

2. Samples of sweet clover *Melilotus albus* Medik. and yellow melilot *Melilotus officinalis* (L.) Pall. with high salt tolerance at early ontogenesis

No. in the VIR catalog	Variety (origin)	Root length, mm (M±SEM)			Root length index IR	
		control	3 atm. NaCl	5 atm. NaCl	3 atm. NaCl	5 atm. NaCl
	S w e e t c l o v e r					
k-15650	Wild (Ivanovo Region)	2.7±0.20	3.4±0.24	2.9±0.21	1.26	1.07
k-35551	Ryadovoi (Primorye Territory)	2.8±0.37	3.3±0.44	2.8±0.72	1.18	0.99
k-38872	Shedevr 75 (Kazakhstan)	1.6±0.22	2.0±0.34	1.4±0.16	1.25	0.88
k-42988	Lutsernovidnyi D-20 (Novosibirsk Region)	3.2±0.59	2.0±0.45	2.5±0.33	1.49	0.79
k-47464	Diomid (Primorye Territory)	2.0±0.38	2.4±0.35	2.2±0.31	1.19	1.07
	Y e l l o w m e l i l o t					
k-38851	Omskii 916 (Omsk Region)	3.5±0.11	3.6±0.17	3.3±0.13	1.02	0.94
k-39362	Novosibirskii 1 (Novosibirsk Region)	3.3±0.20	3.2±0.32	3.0±0.26	0.97	0.91
k-39364	Severotatarskii (Novosibirsk Region)	3.6±0.27	3.5±0.32	2.8±0.25	0.98	0.80
k-40255	Khorogskii 2155 (Novosibirsk Region)	3.0±0.16	3.4±0.19	2.3±0.12	1.13	0.76
k-41031	Omskii 8 (Omsk Region)	2.1±0.19	1.9±0.35	2.0±0.24	0.91	0.95

The chloride salinity, as a rule, led to intensifying the intra- and interspecific variability of melilot sprout growth indexes. It is necessary to notice similar regularity for other plant species under the influence of unfavorable environmental factors. For example, under the drought conditions, variability of some traits among spring wheats was higher [44].

The intraspecific variability of root length of melilot seedlings under salinization increased to a greater degree compared to that of sprouts, which testifies to a higher informative value of the root length index to estimate salt tolerance of melilot and other crops [44]. Linear characteristics of roots are used in studying plant resistance to other abiotic stressors, in particular, to the aluminum toxicity of sour soils [45], drought [46] and low temperatures [47].

The samples tolerant to salinization among the studied sweet clovers and yellow melilots makes 36.2 and 29.6 %, respectively, that is, the adaptation potential of these two species to the excess of sodium chloride in the root zone is approximately identical. Similar results are obtained in Australia while studying the influence of soil salinization on the growth and development of annual and perennial species of melilot introduced there [48].

According to the data obtained in this trial, salinity-resistant species and samples of melilot often originate from regions with widely spread saline soils. This fact must be considered in a strategy of seeking forms valuable for selection for salt tolerance. Perspective varieties may derive from crossing parental forms with high green mass productive and tolerance to salt stress [49]. Despite the available varietal diversity of melilot, the search for new genotypes always remains relevant to create more productive cultivars better adapted to unfavorable environmental factors [50].

Thus, the samples of white and yellow melilot with high initial intensity of plant growth under controlled conditions (in absence of salinization) are for the first time revealed by us in the VIR collections. The presence of sodium chloride in the root zone negatively affects growth indexes of plants. The more severe the stress, the more apparent inhibition of root and sprout growth occurs. Several tested samples and varieties can serve as a new initial material to create yellow and white melilot varieties with tolerance to chloride salinity in early ontogenesis, which will provide better use of their potential for fodder production and bioremediation. Among salinity tolerant varieties that we revealed there are those with high productivity, intensive regrowth after mowing, precocity, bio- and abiotic resistance, and valuable biochemical properties.

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