Evaluation of the resistance of medicago breeding samples to the action of abiotic stressors at the early stages of ontogenesis

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Abstract. New knowledge was obtained about the relationship between the parameters of photosynthesis, transpiration and dark respiration in alfalfa varieties of various ecological and geographical origin at the early stages of ontogenesis, depending on the intensity of abiotic stressors: salinity conditions (0.3 M NaCl) and various intensity of photosynthetically active radiation (from 0 to 1500 mol*$m^{-2}s^{-1}$). Purpose of the work: assessment of the potential resistance of alfalfa varieties and breeding samples to abiotic stressors for breeding for adaptability. To determine the parameters of gas exchange of cotyledon leaves of alfalfa varieties, a portable system for measuring plant gas exchange LI-6800, LI-COR, USA was used. The results of a two-way ANOVA analysis of the influence of the salinity factor and illumination conditions on the resulting signs of gas exchange parameters showed that the proportion of the influence of the genotype of breeding samples on the studied effective signs associated with the operation of the photosynthetic apparatus (the intensity of CO_2 assimilation and the intercellular concentration of CO_2) ranged from 7.25 to 21.35%; associated with water exchange parameters (transpiration and stomatal conductivity for H_2O) varied from 18.80 to 19.84%. Selected breeding samples of alfalfa with high resistance of the photosynthetic apparatus to abiotic stressors.

1 Introduction

To improve the efficiency of modern breeding and seed science, to accelerate the introduction of valuable species into culture, it is necessary to assess the physiological processes that are closely associated with genetic properties [1–3]. The development of physiological diagnostic methods makes it possible not only to assess the state of plants, but also the limits of the modification variability of plants in various conditions of existence in the early stages of development without destroying plant tissues. The development of such methods and approaches makes it possible to conduct studies on the effect of bio- and abiotic stressors on small samples of plants [4-5].

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As important characteristics of CO_2 assimilation, reflecting the conditions of the ecotope and plant species, it is recommended to evaluate the saturation of photosynthetic activity (the maximum intensity of net photosynthesis) and the intensity of dark respiration. The last parameter is most often used to characterize the adaptive capabilities of crops. Dark respiration (Rd) at the plant level is an integral indicator that depends on a whole range of internal and external factors: the stage of ontogenesis, the influence of the ecotope, the physiological state of the studied plant organs, and others. During germination, the intensity of dark respiration increases due to the mobilization of metabolic growth respiration pathways aimed at providing seedlings with energy and plastic substances. Information on the activity and intensity ratio of CO_2 assimilation in cotyledon leaves during seed germination is very limited in the literature, which increases the demand for new knowledge. [6].

Particularly noteworthy are studies on the identification of forms with complex resistance and the creation of new breeding varieties of leguminous grasses on their basis [7].

A collection of original forms of resistant fodder leguminous grasses, which was collected by scientists of the Federal Scientific Center "VIK named after. V.R. Williams" and other scientific institutions is a valuable material both for classical breeding and for new areas of physiological research.

Purpose of the work: assessment of the potential resistance of alfalfa varieties and breeding samples to abiotic stressors for breeding for adaptability.

2 Materials and methods

The objects of research in the experiment were one variety and eight 8 breeding numbers of Medicago L. (*M. varia* Mart., *M falcata* L.) species of different ecological and geographical origin. All these varieties showed high seed and fodder productivity in preliminary field experiments in the collection nursery, were characterized by resistance to a complex of diseases and drought resistance (Table 1).

Table 1. Characteristics of breeding numbers.

Section number	Characteristic	Variety type	View
Agnia	Agnia variety	blue hybrid	*M. varia* Mart.
B–18/14	individual selection from Belgorodskaya 86	blue hybrid	*M. varia* Mart.
B–14/14	individual selection from the variety Manychskaya	blue hybrid	*M. varia* Mart.
B–16/14	individual selection from variety Marusinskaya 425	yellow hybrid	*M. varia* Mart.
B–35/17	individual selection from the variety Krasnoyaruzhskaya-2	blue hybrid	*M. varia* Mart.
B–12/14	individual selection from Belgorodskaya 86	blue hybrid	*M. varia* Mart.
B–17/14	individual selection from Belgorodskaya 86	blue hybrid	*M. varia* Mart.
B–26/14	individual selection from variety Vega 87	blue hybrid	*M. varia* Mart.
B–33/14	individual selection from variety Pavlovskaya 7	crescent (yellow)	*M falcata* L.

The experiments were carried out on the cotyledon leaves of alfalfa, since the physiological processes in the cotyledon leaves, as the first photosynthetic organs of a germinating plant organism, are a reflection of the metabolic potential of the embryo.

As a substrate, a mixture of peat, perlite, sand, and an organic soil mixture was used in a ratio of 2:3:3:4 by volume in accordance with the procedure [8-9]. Scarified seeds of breeding samples and varieties of alfalfa of various origins were germinated in 150 ml vessels in a climate chamber. Imitation of salinity conditions was carried out according to the methods adopted in physiological studies: control - water (H_2O), experiment - salinization with 0.3 M NaCl. The concentration of 0.3 M NaCl for a series of experiments

was chosen as a result of preliminary experiments to develop a methodology for assessing the resistance of breeding samples to salinity. The repetition in the experiment is 6-fold. The vessels were placed in a climatic chamber at a temperature of 24°C.

The study of the processes of photosynthesis, dark respiration, and transpiration was carried out on cotyledon leaves using a portable system for measuring plant gas exchange (model LI-6800, LI-COR, USA) according to the method described in section 2.2.4. The intensity of CO_2 assimilation (A, μmol*m^{-2}s^{-1}), transpiration intensity (E, mol*m^{-2}s^{-1}), stomatal conductivity of leaves for water vapor (gsw, mol*m^{-2} s^{-1}) and intercellular CO_2 concentration (Ci, μmol*mol^{-1}). Cotyledon leaves were placed in a thermostatically controlled leaf chamber of a portable system for measuring plant gas exchange (model LI-6800, LI-COR, USA) (the device was purchased with the financial support of a grant for the creation of a breeding and seed center of the Federal Scientific Center "VIC named after V.R. Williams"). The intensity of photosynthetically active radiation (PAR) in the chamber varied in the sequence: 1500, 1000, 500, 200, 100, 50, 0 μM*m^{-2}s^{-1}. The value of dark respiration (Rd) was determined from the value of CO2 assimilation in the absence of illumination [10-11].

Statistical analysis of the results of laboratory experiments was carried out using the Excel application package, ANOVA analysis. The obtained averaged data were processed by the method of analysis of variance (ANOVA) of two-factor complexes with the determination of the strength of influence (h^2x) of various factors, as well as the least significant difference ($LSD_{0.05}$) between the options [12].

3 Results and Discussion

As a result of a series of laboratory experiments, the studied alfalfa breeding samples were divided into two groups depending on the degree of stability of the photosynthetic apparatus and gas exchange parameters to stress factors: "stable" and "unstable" forms "stable" and "unstable".

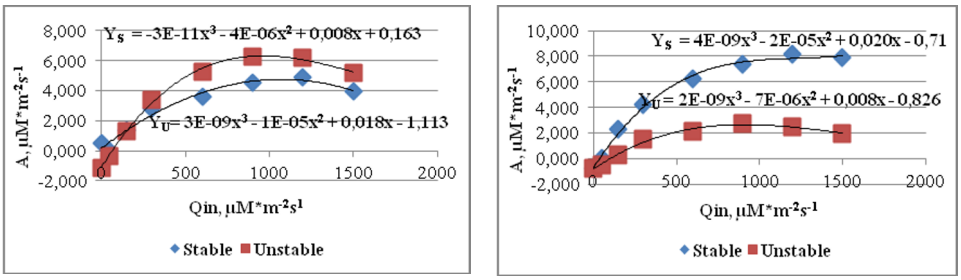
Three breeding numbers - B-18/14, B-35/17 and B-33/14 were characterized by the stability of the photosynthetic apparatus and transpiration parameters under saline conditions and were assigned to the group conventionally called "resistant". In breeding alfalfa samples of this group, both in the variant with the introduction of 0.3 M NaCl into the medium, and in the control variant (H_2O), photosynthesis light saturation occurred at a PAR intensity of 1200-1500 μM*m^{-2}s^{-1}. The intensity of CO_2 assimilation in seedlings in the 0.3 M NaCl variant compared to the control variant was higher by 33.4–198.4%; intercellular CO_2 concentration - by 1.9–13.58%; the intensity of transpiration - by 23.97–126.1%, the value of stomatal conductance for H_2O – by 24.71–131.9%.

The intensity of dark respiration in the variant with salinity was lower by 12.75–104.5% in breeding accessions B–18/14, B–35/17, and B–33/14 compared to the control.

The second group - "unstable" - included alfalfa varieties, in which the intensity of photosynthesis during salinization significantly decreased, and inhibition of water exchange processes was also noted: variety Agnia, B–14/14, B–16/14, B–12/14, B -17/14, B-26/14. In the seedlings of these variety samples, light saturation of photosynthesis occurred at a PAR intensity of 900-1200 μM*m^{-2}s^{-1}. The intensity of photosynthesis under saline conditions decreased by 60.2–75.1% compared to the control variant; intercellular CO_2 concentration - by 15.02–19.64%; intensity of dark respiration - by 56.8%; transpiration intensity - by 13.1–70.69%; the value of stomatal conductivity for H_2O - by 21.07–70.88%.

In order to identify interpopulation differences between stable and unstable groups of breeding samples, a comparative analysis was carried out for the combined groups of "stable" and "unstable" forms "stable" and "unstable". Light curves of photosynthesis in cotyledon leaves of alfalfa depending on salinity resistance, with a high coefficient of curve

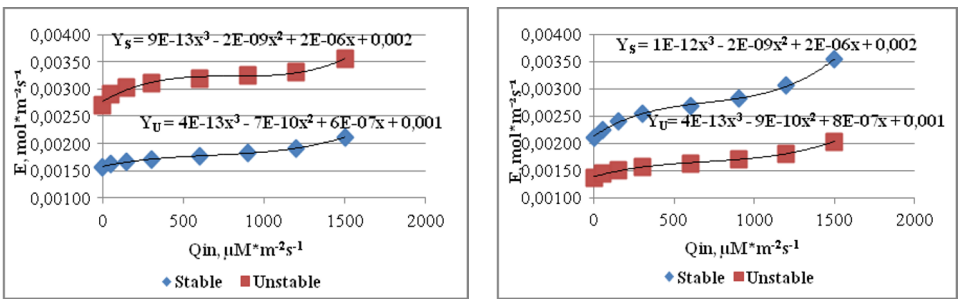
approximation (R^2 = 0.965-0.997 in the control variant and R^2 = 0.990-0.994 in salinity) are described by third-order polynomial equations (Figure 1)



A B

Fig. 1. Dynamics of CO_2 assimilation intensity ($\mu M*m^{-2}s^{-1}$) in alfalfa cotyledon leaves with different salinity tolerance depending on PAR intensity: A - control variant (H_2O); B - experimental variant (0.3 M NaCl).

Light curves of transpiration intensity in alfalfa cotyledon leaves depending on salinity resistance, with a high curve approximation coefficient (R^2 = 0.976-0.996 in the control variant and R^2 = 0.997 in salinity) are described by a third-order polynomial equation (Figure 2).



A B

Fig. 2. Dynamics of transpiration intensity (mol m^{-2} s^{-1}) in alfalfa cotyledon leaves with different salinity tolerance depending on PAR intensity (H2O control): A - control variant (H_2O); B - experimental variant (0.3 M NaCl).

The results of a two-way dispersion analysis of the effect of salinity factors and lighting conditions on the resulting signs of gas exchange parameters in seedlings of alfalfa breeding samples grown on a peat substrate made it possible to establish a significant effect of the studied factors: factor A "salinity conditions", as well as factor B "light intensity" on the studied effective signs depending on the genetic component (Table 2).

Factor A (salinity conditions) significantly affects the resulting signs "photosynthesis intensity" (h^2_x=3.27%); "Transpiration intensity" (h^2_x=17.13%); and “stomatal conductivity value for H_2O” (h^2_x=17.81%). Factor B (illumination intensity) had a significant impact at the level of 43.63% on the effective feature "CO_2 assimilation intensity".

No significant influence of the studied factors on the resultant sign "intercellular concentration of CO_2" has been established. At the same time, it was revealed that the share of random factors in the total variance for this trait is 75.44%.

Table 2. The results of a two-way ANOVA analysis of the effect of salinity and PAR intensity on the studied indicators of gas exchange in selection samples of alfalfa.

Effective sign	Source of Variation	D	n-1	s^2	F_f	$F_{st0.05}$	h^2_x
A, μM*m^{-2}s^{-1}	General	999.54	143				100
	Random	442.72	120	3.68			44.29
	Sample genotype	72.51	8				7.25
	A	32.78	1	32.78	8.88	3.9	3.27
	B	436.06	7	62.29	16.88	2.1	43.62
	A×B	15.462	7	2.21	0.59	3.3	1.54
Ci, μmol*mol^{-1}	General	6195668	159				100
	Random	4673863	135	34621.2			75.43
	Sample genotype	1323229	9				21.35
	A	17284.8	1	17284.8	0.49	8.6	0.279
	B	179928	7	25704	0.74	3.3	2.90
	A×B	1363.42	7	194.77	0.006	3.3	0.02
E, mol*m^{-2}s^{-1}	General	0.00011	143				100
	Random	6.53E-05	120	5.44E-07			59.22
	Sample genotype	2.07E-05	8				18.80
	A	1.89E-05	1	1.89E-05	34.71	3.9	17.13
	B	5.25E-06	7	7.51E-07	1.39	2.1	4.76
	A×B	7.83E-08	7	1.12E-08	0.025	3.3	0.0709
gsw, mol*m^{-2} s^{-1}	General	0.098538	143				100
	Random	0.058444	120	0.00048			59.31
	Sample genotype	0.019	8				19.84
	A	0.017	1	0.0175	36.04	3.9	17.81
	B	0.0029	7	0.00041	0.845	3.3	2.92
	A×B	0.00011	7	1.46E-05	0.030	3.3	0.13

Note: D is the sum of squared deviations (deviant); s^2 - dispersion; n-1 is the number of degrees of freedom; h^2x is the power of influence on the effective attribute. F_f is the actual value of Fisher's F-test; $F_{0.05}$ - tabular value of F - Fisher's test at a significance level of 5%; factor A – "salinity conditions"; factor B - "light intensity"

4 Conclusion

As a result of the studies, three breeding forms of alfalfa were identified that are potentially resistant to salinity conditions at the early stages of ontogenesis: they are characterized by an increase in the intensity of photosynthesis and water exchange parameters against the background of a decrease in the intensity of dark respiration.

A two-way dispersion analysis of the results of the experiment showed that the factor "salinity conditions" significantly affects all the effective features. The results obtained indicate the impossibility of rejecting the null hypothesis of the influence of the factor on most of the resulting traits, while they have the greatest impact on the intensity of CO_2 assimilation and allow us to state the fact of a significant dependence of photosynthesis, transpiration and intercellular CO_2 concentration on salinity conditions. In the total dispersion of the studied effective signs, the share of the influence of random factors was at a fairly high level - 44.3-75.4%.

A significant influence of the genotype of the studied breeding samples on resistance to abiotic factors - salinity and PAR intensity - has been established. The share of the influence of the genotype of alfalfa breeding samples on the complex effective traits associated with the operation of the photosynthetic apparatus was 7.25–21.35%; on the studied effective features associated with the intensity of water exchange varied from 18.80 to 19.84%.

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