AgroLife Scientific Journal - Volume 9, Number 2, 2020
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

EVALUATION OF DROUGHT TOLERANCE IN ALFALFA (*Medicago sativa*) GENOTYPES IN THE CONDITIONS OF OSMOTIC STRESS

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Abstract

The aim of the study was to evaluate alfalfa material on drought tolerance to distinguish the genotypes, which are resistant to water deficit at early stages of growth. The study was carried out during 2016-2018 in laboratory conditions at the Institute of Irrigated Agriculture of NAAS (Ukraine) using alfalfa seeds of different genotypes were subjected to germination in sucrose osmotic solutions with a pressure of 0.3, 0.5, 0.7 MPa within 7 days in the thermostat at the temperature 20 ± 2ºC. The control variant - purified water. The highest germination rate of alfalfa seeds was observed in the purified water - 82.0-96.1%. The increase of osmotic pressure in sucrose solutions decreased germination rate of seeds. Root length and hypocotyl length reached the highest values in the purified water – 24.1-33.9 and 26.1-34.1 mm, respectively. Sucrose osmotic solution with the pressure of 3.04 bar oppressed the growth of root length and hypocotyl length, which at this variant reached 11.4-19.9 and 13.8-21.9 mm, respectively. Besides, different alfalfa genotypes showed different reaction to the osmotic stress. The highest values of vegetation growth intensity (VI) in the osmotic solution with the pressure of 0.3 MPa were recorded for genotypes MgP and Unitro - 31.7 and 34.1, respectively. The values of the index at 0.5 MPa decreased in the genotypes to 19.4 and 17.6, respectively. Particular attention should be paid to the genotypes Kazachka No.2 and Pr, which are characterized with a gradual decrease in VI with the increase of osmotic pressure from 0.3 MPa to 0.7 MPa in the solution: 20.3, 13.1, 10.5 and 25.1, 19.9, 7.3, respectively. The genotypes Kazachka No.2, Pr, Unitro had the best indices of germination rate, root, and hypocotyl length, VI values, and are recommended as the most prospective in alfalfa breeding for drought tolerance.

Key words*: alfalfa, drought tolerance, sucrose, seed, germination rate, vegetation growth intensity, hypocotyl, embryonic root.*

INTRODUCTION

Recent increase in the warmth of climate resulted in the increase in adverse and extreme factors occurrence frequency. Firstly, it is an intensive raise of mean monthly air temperature, a decrease in rainfall amounts under the uneven distribution of precipitation, and prolonged drought period in the summertime (Lykhovyd, 2018). As a result, it is difficult to obtain strong sprouts of crops, which suffer from water stress and are oppressed, that results in the yield losses.

Therefore, the study of alfalfa genotypes adaptability to the adverse conditions of environment are of a great value, besides, it is also important to study various breeding material of alfalfa to create adaptive varieties with sustainable productivity.

Some scientists consider the ability of crops to deal with adverse environmental conditions during the vegetation season and form yield at the expense of biological features and genotype peculiarities is mainly connected with their drought tolerance (Orliuk & Honcharova, 2002).

Therefore, getting high alfalfa yields in arid conditions relates to seeking and creation of new morphotypes and adaptive potential. The most widely spread method for the evaluation of drought tolerance is water stress modeling using solution with high osmotic pressure. Greater number of sprouts testify about the ability of a variety to use low moisture content from soil that is an indirect indicator of its drought tolerance. Therefore, we can predict the resistance of genotypes to water stress at early stages (Udovenko, 1976; Gharoobi et al., 2012) and distinguish the populations with a stress-tolerance at the earliest stages of growth (Sfarnejad, 2008; Hamidi & Safarnejad, 2010; Moskalets & Rybalchenko, 2015; Bychkova &

Khlebova, 2015; Kornievskaya & Silantjeva, 2018). Using different osmotic materials is one of the best methods of studying the effect of osmotic stress on the sprouting (Gharoobi et al., 2012). Prediction of drought tolerance of crops could be made using seed sprouting in the sucrose solutions (Denisov & Osipova, 2013), polyethylene glycol (PEG) (Budakli & Erdel, 2015; Özkurt et al., 2019) or NaCl solutions (Lavrenko et al., 2019) if it is necessary to assess the tolerance to soil salinity level. This method has valuable advantages: simplicity, affordability, low labor expenditures, independence on weather conditions, and a possibility of conduction all year long. Seeds, which are cultivated in osmotic solutions, imitate water stress due to the fact of taking water from living cells by saccharose. Drought tolerant plants have high water holding capacity. Therefore, the greater number of seeds germinate in the solution, the more drought tolerance the plant has. It is considered that using sucrose solutions of different concentrations for the evaluation of drought tolerance is not only of theoretical, but of practical value either.

The intensity of sprouts growth in the conditions of higher osmotic pressure of water solution characterizes the capacities of a genotype to survive in extreme conditions. The capacity of seed to give strong sprouts in the conditions of «physiological drought» tells us about the ability of seeds to germinate under the conditions of low moisture content and their high water uptake force, which determines the volume of water that might be consumed from solutions. This approach allows concluding seed germination rate and control the process of plants resistance to water deficit at the earliest stages of ontogenesis. It was determined that species and varieties differ by seeds germination in the stress conditions (Budakli & Erdel, 2015; Molor et al., 2016).

One of the most important stages of plant breeding process is a preliminary evaluation of drought tolerance of a large set of populations to choose the best primary material. Right choice of primary material is a pre-condition of further success in plant breeding.

Sucrose concentration for seed germination evaluation of alfalfa was selected to differentiate plant breeding material by the feature of drought tolerance. The level of expression of the studied feature is evaluated by the number of seeds that had germinated.

The aim of the study was the evaluation of primary alfalfa material on drought tolerance at different concentrations of sucrose in laboratory conditions, and find out the populations, which are resistant to water deficit at early stages of growth to use the obtained data in further plant breeding work.

MATERIALS AND METHODS

The study was carried out using sucrose solutions with the osmotic pressure of 0.3, 0.5, 0.7 MPa. To obtain the mentioned solutions, we dissolved 4.3, 7.2 and 10.0 g of sucrose in 100 ml of purified water. The control variant was purified water. Alfalfa seeds were germinated in Petri dishes, in which pieces of filter paper were placed. The dishes and paper were sterilized in a thermostat at the temperature +105°C for 4 hours, the solutions were sterilized in autoclave at the pressure of 1 MPa for 0.5 hours. We used undamaged seeds of alfalfa varieties Unitro, Veselka, populations Kazachka No.2, Pr, MgCP-11, Ram.d, MgP, LRH, Ver.d, Med, Dk of the reproductions obtained in 2016, 2017 and 2018. The seeds of every sample were placed in 4-layer gauze bags and sunk into the solution of antiseptic (1% solution of $KMnO_4$) for 10 minutes. Then they were washed with fresh-water and dried on the filter paper, evenly distributed in the dishes by 50 in each dish. The dishes were closed with caps and placed into a thermostat at the temperature 20±2°C for seven days. Every day the number of germinated seeds was recorded. The experiment was carried out in four replications. The percentage of germinated seeds was calculated as follows (1):

$$P = a/b \times 100,\% \quad (1)$$

where: a - mean number of seeds, which germinated in the sucrose solutions, b - mean number of seeds, which germinated in the purified water (control).

The level of drought tolerance was determined by the number of seeds, which germinated in the sucrose solutions.

However, germination rate of the seeds is insufficient for the drought tolerance assessment. That is why the index of vegetation growth intensity (VI) was additionally determined. We took into account the peculiarities and intensity of the roots and hypocotyl growth as a more informative index for the assessment of sowing qualities of the seeds (Karpin et al., 2012; Budakli & Erdel, 2015; Özkurt et al., 2019). The vegetation growth intensity (VI) was determined using the formula (2):

$$VI = RL + SL \, x \, GP/100, \qquad (2)$$

where: RL - the length of embryonic root, mm; SL - the length of hypocotyl, mm; GP - germination percentage, % (Abdul-baki & Anderson, 1970).

Statistical evaluation of the results was carried out at $p<0.05$ within Microsoft Excel software performing Fisher's least significant difference (LSD) test for comparison of the variants (Dospekhov, 1985; Ushkarenko et al., 2008; Williams & Abdi, 2010).

Variation was calculated by the standard methodology as the ratio of the standard deviation to the mean value for the data set (Everitt & Skrondal, 2002).

RESULTS AND DISCUSSIONS

The analysis of the experimental results testifies that germination rate of alfalfa seeds in the purified water (control) depending on the population and variety, fluctuated within 82.0-96.1%. The highest germination rate was recorded for Dk (96.1%), Unitro (95.0%), MgCP-11 (93.6%), Veselka (90.0%). The lowest germination rate in the control variant was recorded for the population Ver.d., with the germination rate of 82.0%.

Germination of the alfalfa seeds in the control variant started on the next day, the maximum energy of germination was observed on the third day. Germination was significantly delayed in the osmotic solutions, where single seeds germinated just beginning from the second-third day (Figure 1).

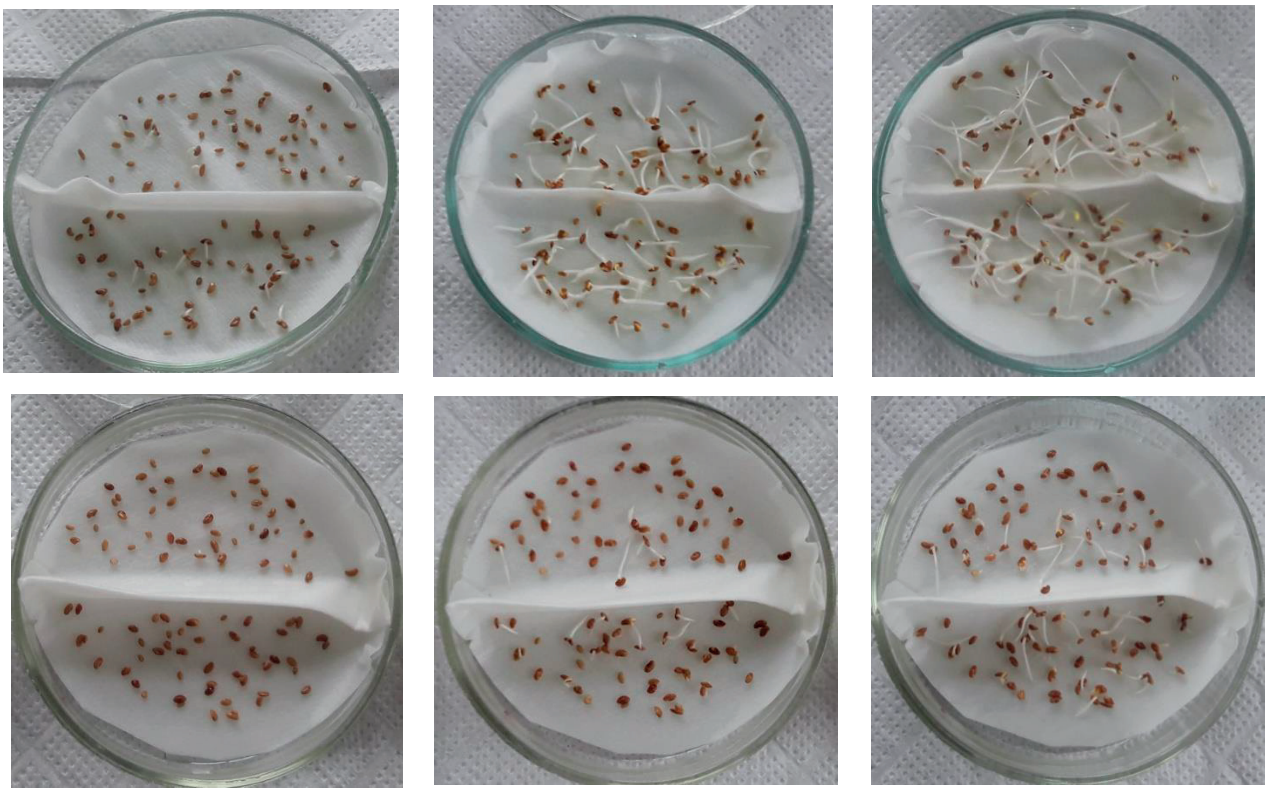

Figure 1. Germination of alfalfa seeds at 1st, 2nd, 3rd day (upper row - control, lower low - sucrose - 0.3 MPa)

Our results are confirmed by the reports, claiming that the increase of osmotic pressure leads to the increase in time required for germination of alfalfa seeds, and at the maximum concentrations this time increases 2.4-2.5 times depending on the crop genotype (Tilaki et al., 2009; Ghasemi & Shah, 2016).

The germination percentage decreased gradually with the increase of sucrose concentration in comparison to the control. The minimum was reached at 0.7 MPa, while some of the studied genotypes showed the minimum just at 0.5 MPa. However, some of the studied genotypes showed almost equal to the control germination ability even at the osmotic pressure of 0.3-0.5 MPa. For example, genotypes Pr, Ver.d had germination percentage of 85.6 and 82.0% in the control variant, and under the osmotic stress of 0.3 MPa - 83.0 and 78.0%, and under the pressure of the osmotic solution 0.5 MPa - 79.6% and 72.0%, respectively. The variety Unitro preserved high ability to germinate under the minimum (0.3 MPa) osmotic stress - 82.6% (Figure 2).

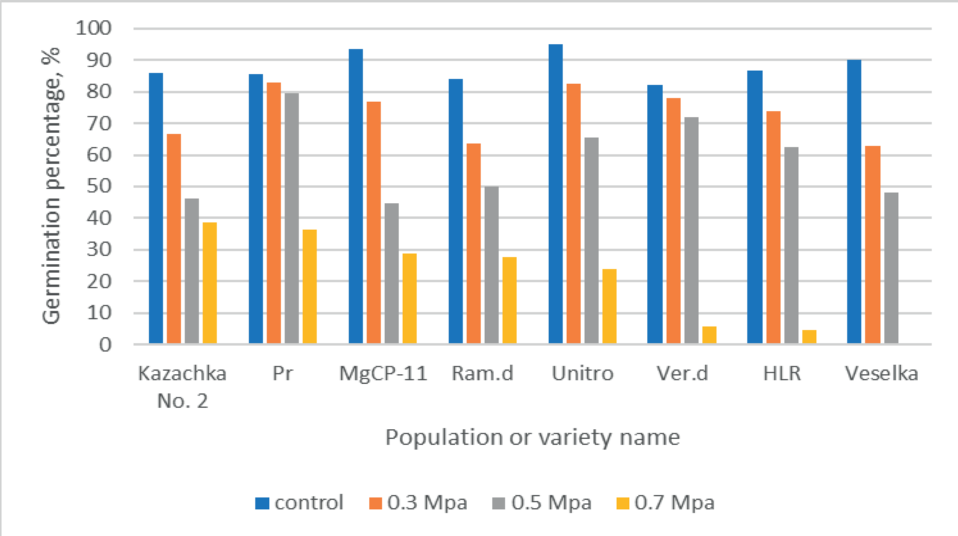

Figure 2. Germination rate of alfalfa at different concentrations of osmotic solution (average for 2016-2018)

The decrease in germination rate of different alfalfa varieties under the impact of osmotic stress has been determined by the number of scientists (Castroluna et al., 2014; Özkurt et al., 2019). Their results testified that the minimum stress does not have significant effect on germination, and the greater concentration of osmotic solutions were, the lower germination rate of alfalfa seeds was, that agrees with our results. Some researchers reported about the fact that the lowest (1.4 MPa) studied osmotic pressure significantly oppressed the germination of *Triticeae* seeds, while at the osmotic pressure of 2.0-2.4 MPa it was rapidly decreasing. Germination rate of seeds is the most informative index for the differentiation of crops by their sensitivity to water stress in laboratory studies (Budakli & Erdel, 2015).

Our research shows that the lowest germination rate is at the highest osmotic pressure of 0.7 MPa - germination percentage fluctuated within 0-38.6%. The maximum root and hypocotyl lengths were recorded for the control variant with purified water with the fluctuations within 24.1-33.9 and 26.1-34.1 mm, respectively (Table 1).

Table 1. Effect of the osmotic stress on the sprouts of the alfalfa genotypes (average for 2016-2018)

Name of a population	Root length, mm				Length of the hypocotyl, mm			
	control	3.04 bar	5.07 bar	7.09 bar	control	3.04 bar	5.07 bar	7.09 bar
Kazachka No. 2	25.7	14.2	13.2*	12.5*	27.4	16.2	15.2*	14.5*
Pr	30.4	14.1	11.0	10.0*	31.0	16.1	14.0*	10.0*
MgCP-11	28.6	11.4	10.7	0.0	29.4	13.8	12.8	0.0
Ver.d	32.3	17.5*	11.2	0.0	33.5	19.4	12.3	0.0
MgP	33.9	19.8*	14.4*	0.0	34.1	21.9*	17.6*	0.0
HLR	24.1	13.5	5.5	0.0	26.1	15.5	6.9	0.0
Unitro	30.0	19.9*	12.5*	0.0	32.2	21.4*	14.3*	0.0
Average by a population	29.2	15.8	11.2	3.2	30.5	17.8	13.3	3.5
Variation,%	60.3	42.2	48.1	61.9	57.4	40.1	47.1	60.6
S_x	3.6	2.54	2.66	2.69	3.7	2.59	2.76	2.75
LSD_{05}	8.3	5.80	6.10	6.10	8.4	5.9	6.32	6.29

Note: *- significant at p<0.05; source: own study.

The osmotic potential of 0.3 MPa started to cause inhibitory effect on the roots and hypocotyl growth, although, the length of root and hypocotyl in the variant was higher than on other studied sucrose solutions - 11.4-19.9 mm for root, and 13.8-21.9 mm for hypocotyl, respectively. The best values of the indices were recorded in the population MgP and variety Unitro (19.8, 19.9 and 21.4, 21.9 mm, respectively).

We should mention that the increase in osmotic potential decreases the root length, but the reaction of different genotypes is different for every studied sucrose concentration. The populations Kazachka No. 2 and Pr slowly decrease the length of sprouts with a gradual increase in the osmotic pressure from 0.3 to 0.5 and 0.7 MPa. Other populations demonstrated critical decrease in the growth at the osmotic stress of 0.3 and 0.5 MPa, and at the stress of 0.7 MPa their growth was severely oppressed, resulting in the root length, which is equal to the seed length, or its length was 10 mm without hypocotyl formation.

Osmotic stress has a great effect on the root and hypocotyl lengths (Castroluna et al., 2014; Özkurt et al., 2019). The study of Budakli & Erdel (2015) showed that they reached the peak length at the osmotic pressure of 2.95 bar, while further increase resulted in the sharp decrease of the root and hypocotyl lengths. Osmotic solution (1.6 MPa) significantly limits growth processes of *Triticeae* plants. The critical osmotic pressure for the coleoptile growth of such plants is 2.0 MPa, when it is weakly growing and forms the length of just about 0.2-0.8 cm or does not appear at all (Moskalets & Rybalchenko, 2015). The increase in osmotic pressure of solutions resulted in the oppression of root growth in the plants (Varavkin & Taran, 2014).

The increase in osmotic stress increases variation of the root length (V= 42.2-61.9%) and hypocotyl length (V= 40.1-60.6%).

We also have determined the VI values. As some scientific reports testify, the level of VI is a good estimator for the potential of further growth (Varavkin & Taran, 2014; Özkurt et al., 2019).

The highest VI in our study was recorded in the control variant. The reaction on the osmotic stress was different in the studied populations and varieties of alfalfa. The highest VI at the 0.3 MPa osmotic stress was recorded for the population MgP and variety Unitro - 31.7, 34.1 and 19.4, 17.6, respectively (Table 2).

Table 2. Vegetation growth intensity (VI) of the sprouts of the alfalfa genotypes (average for 2016-2018)

Name of a population	Vegetation index of the sprouts at different osmotic pressure			
	control	3.04 bar	5.07 bar	7.09 bar
Kazachka No. 2	45.7	20.3	13.1	10.5*
Pr	52.6	25.1	19.9*	7.3
MgCP-11	54.3	19.4	10.5	0.0
Ver.d	55.3	23.4	11.8	0.0
MgP	59.8*	31.7*	19.4*	0.0
HLR	43.6	21.5	7.8	0.0
Unitro	59.1*	34.1*	17.6*	0.0
Average by a population	51.4	21.5	14.3	2.5
Variation, %	67.3	46.5	59.6	73.4
S_x	7.0	4.8	5.4	5.4
LSD_{05}	12.1	7.1	8.3	8.5

Note: *significant at p<0.05; source: own study.

Low VI was attributed to the populations MgCP-11 and HLR, the greatest decrease in the index was observed at the osmotic pressure of 0.5 MPa (10.8 and 7.8, respectively). The populations Kazachka No. 2 and Pr are characterized with slight gradual decrease in VI with the increase in osmotic stress (20.3, 13.1 and 25.1, 19.9 for 0.3 and 0.5 MPa, respectively) with the minimum values of the index at the pressure of 0.7 MPa - 10.5 and 7.3, respectively.

The studies of Varavkin & Taran (2014), Özkurt et al. (2019) support our results, testifying about gradual decrease in VI of plants with the increase in osmotic stress.

CONCLUSIONS

Laboratory study on the determination of alfalfa seeds germination rate and initial growth peculiarities under the osmotic stress, conducted using sucrose solutions with the osmotic pressure of 3-7 MPa, allowed screening plant breeding material for the drought tolerance and determination of the populations Kazachka No. 2, Pr, and variety Unitro with the highest indices of germination rate and vegetation growth intensity as the best ones for further selection work on obtaining highly resistant to drought varieties of the crop.

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