THE BEHAVIOR OF SOME ROMANIAN ALFALFA GENOTYPES TO SALT AND WATER STRESS

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ABSTRACT

Abiotic stress conditions cause extensive losses to agricultural production worldwide (Bray, 2002). Drought and salinity stress can significantly affect plant yield in arid and semi-arid regions and not only. Climatic changes will conduct to severe drought conditions and to aridity of some important regions in Romania. One of the most important strategies which could reduce the influence of drought and salinity on alfalfa (Medicago sativa) production is to breed for increased cultivar tolerance. The present paper reports the reactions of some Romanian alfalfa genotypes to salt and water stress. The aim was to elucidate some physiological and metabolic aspects of those stresses, in order to establish screening criteria to facilitate the development of genotypes with enhanced tolerance to field stress conditions. Seeds of nine alfalfa genotypes were sown in Mitterlich plots filled with a soil-sand mixture. The plant were grown in vegetation house under optimal condition up to just before flowering, when for water stress variant the watering was reduced for 10 days; salt stress was imposed on plants by adding 300 mM NaCl/l and under combined stress the plants were treated with 300 mM NaCl/l one week before reducing watering. The alfalfa yield of all studied genotypes was significantly reduced under water and salt stress while stresses combination caused a reduction on fresh biomass, too. Salt stress significantly decreased biomass by more than 37% while water stress by more than 73%. The effects of salt and water stresses on yields were additive but not equal. Alfalfa responded to drought by decreasing leaves transpiration. Between biomass accumulation and leaves transpiration under water and salt stress there was a linear relationship ($r = 0.76^*$; $r = 0.82^*$). Under optimal condition the proline content was very small (1.7-5.4 mg proline/g f.w.) but there were obviously higher proline contents under salt stress (156-441 µM proline/g f.w.), water stress (45-68 µM proline/g f.w.) and stress combination (120-330 µM proline/g f.w.). The negative effect of salinity and combined stresses on alfalfa growth could be attributed to osmotic effects. Osmotic stress inhibits water uptake from the soil and requires the plant to use energy and carbohydrates in synthesizing organic solutes to adjust its internal osmotic potential. Yield loss results from closing stomata and from energy and carbohydrates use in osmoregulation. The leaves transpiration and biomass accumulation were correlated, suggesting the use of transpiration as a screening tool for drought and saline tolerance of alfalfa genotypes.

Key words: alfalfa, biomass, drought, leaves transpiration, proline content, salinity.

INTRODUCTION

Abiotic stress conditions cause extensive losses to agricultural production worldwide (Bray, 2002). Drought and salinity stress can significantly affect plant yield in arid and semi-arid regions and not only. Climatic changes will conduct to severe drought conditions and to aridity of some important regions in Romania. Knowledge of the physiological mechanisms underlying the response to these abiotic stresses is important for understanding of stress tolerance and for germplasm improvement. The present paper reports the reactions of some Romanian alfalfa genotypes to salt and water stress. The aim was to elucidate some physiological and metabolic aspects of those stresses, in order to establish screening criteria to facilitate the development of genotypes with enhanced tolerance to field stress conditions.

MATERIAL AND METHODS

The study was conducted in a vegetation house at NARDI Fundulea, in 2006. Nine alfalfa genotypes were grown in soil: sand mixture in Mitterlich plots under optimal watering regimes up to beginning of flowering.

After this period under control treatment plants were maintained in the same conditions, under water stress treatment watering was reduced for 10 days; salt stress was imposed on plants by adding 300 mM NaCl/l and under combined stress plants were treated with 300 mM NaCl/l one week before reducing watering. The following determinations were made:

Yield was estimated by measuring biomass accumulation of aerial part (shoots and leaves) (fresh weight).

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**Leaves transpiration** each replicate was constituted by five pair of trifoliate leaves, which were weighed, maintained under lab conditions for five hours, again weighed and then dried into oven for 12 hours. The leaves transpiration was calculated according to Clarke et al. (1991) formula.

**Proline content**: proline was determined by spectrophotometry following the ninhydrin method described by Bates et al., (1973) using L-proline as a standard. Approximately 0.5 g of fresh leaves was homogenized in 10 mL of 3% aqueous sulphosalicilic acid and filtered. To 2 mL of the filtrate, 2 mL of acid ninhydrin was added, followed by the addition of 2 mL glacial acetic acid and boiling for 60 min. The mixture was extracted with toluene and free proline was quantified by spectrophotometry at 520 nm.

**RESULTS AND DISCUSSION**

The analysis of variance regarding the effect of drought and salinity on alfalfa biomass showed a very significant influence of treatments, genotypes and their interaction, but the variance of treatments was higher than the variance due to genotypes (Table 1).

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>DF</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>79572.34</td>
<td>26524.1</td>
<td>4270.6***</td>
</tr>
<tr>
<td>Error A</td>
<td>6</td>
<td>37.265</td>
<td>6.211</td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td>8</td>
<td>1164.823</td>
<td>145.60</td>
<td>603.03***</td>
</tr>
<tr>
<td>Interaction</td>
<td>24</td>
<td>2522.716</td>
<td>105.11</td>
<td>435.34***</td>
</tr>
<tr>
<td>Error B</td>
<td>64</td>
<td>15.453</td>
<td>2415</td>
<td></td>
</tr>
</tbody>
</table>

For all genotypes, the biomass obtained at each treatment was significantly different from the control and the general trend was to more pronounced decrease under drought and combined stress.

Salt stress significantly decreased biomass by over 37% while water stress by over 73%. The effects of salt and water stress on yields were additive but not equal (Figure 1). A decrease in biomass accumulation due to drought and salinity was associated with decreased transpiration. The genotypic differences in water loss through transpiration are obvious.

Cultivars Sandra, Cosmina and Dorina had relatively low leaves transpiration while the cultivars Dana and F 130T, had highest water loss, under both control and stress conditions. The effects of salt and water stress on leaves transpiration were additive, but not equal (Figure 2).

![Figure 1. The effects of stresses on biomass accumulation in studied alfalfa genotypes](image1)

![Figure 2. The effects of stresses on leaves transpiration in studied alfalfa genotypes](image2)

The alfalfa biomass was linearly related to transpiration and the values of the correlation coefficients between biomass and transpiration under water and salt stress were \( r = 0.82 \) and \( r = 0.76 \) (P<0.01) (Table 2).

Under optimal condition the proline content was very small (1.7-5.4 mg proline/g f.w.), but...
was obviously higher under saline stress (156-441 
µM proline/g f.w.), water stress (45-68 µM 
proline/g. f.w.). The effects of the two stresses 
appeared to be additive, but not equal (120-330 
µM proline/g f.w.) (Figure 3).

There was a correlation between biomass 
and proline content only under drought and combi-

ded stress (Table 2). This suggests that proline 
accumulation is necessary under salt stress, but 
this is not enough to give tolerance to salinity.

**Table 2. Relationship between biomass and leaves transpiration under stress conditions**

<table>
<thead>
<tr>
<th>Specification</th>
<th>Transpiration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drought</td>
</tr>
<tr>
<td>Biomass</td>
<td>r = 0.82*</td>
</tr>
<tr>
<td></td>
<td>y = 0.0643x – 0.5117</td>
</tr>
<tr>
<td>Proline content</td>
<td>r = -0.64*</td>
</tr>
<tr>
<td></td>
<td>y = -3.3x + 103.44</td>
</tr>
</tbody>
</table>

The negative effect of salinity and combined 
stress on alfalfa growth could be attributed to an 
osmotic effect. Osmotic stress inhibits water up-
take from the soil and requires the plant to use 
energy and carbohydrates in synthesizing organic 
solutes to adjust its internal osmotic potential.

The significance of proline accumulation in 
osmotic adjustment is still debated and varies ac-
cording to the species. Ashraf (1989), Lutts et al. 
(1996), Feitosa de Lacerda et al. (2001) and 
Meloni et al. (2001) reported that proline is not 
involved in the osmotic adjustment of black gram, 
sorghum, rice and cotton cultivars, respectively. 
Heuer (2003) reported that proline was not able 
to counteract salt stress effects in salt-sensitive 
tomato plants but the osmolytes (proline, glycine, 
phenol etc.) are considered to stabilize proteins 
and cellular structure and can increase the osmotic 
pressure of the cell (Yancey et al., 1982). This 
response is homeostatic for cell water status and 
protein integrity, which are perturbed in the face 
of the soil solutions containing higher amounts of 
NaCl and the consequent loss of water from the 
cell.

**CONCLUSIONS**

Our results established the negative effects of 
water and salt stresses on biomass accumulation 
and leaves transpiration and positive ones on 
proline accumulation. Yield loss results from clos-
ing stomata (to decrease the transpiration) and 
from increasing energy and carbohydrate use in 
osmoregulation.

The leaves transpiration and biomass accumu-
lization were correlated, suggesting that the for-
mer might be used as screening criterion for 
drought and saline tolerance of alfalfa genotypes.

In comparison with evaluation and selection 
for alfalfa drought and salt tolerance under field 
conditions, this is a simple and efficient method of 
screening during the first year of growth and ear-
lier stages of vegetation, when alfalfa is very sensi-
tive to this kind of stresses.

**REFERENCES**


