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 Mitcherlich 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 Mitcherlich 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 than 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 ninhidrin 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 1 . Analysis of variance for biomass

Source of variance	DF	Sum of squares	Mean square	F value
Treatment (stresses)	3	79572.34	26524.1	4270.6***
Error A	6	37.265	6.211	
Genotypes	8	1164.823	145.60	603.03***
Interaction	24	2522.716	105.11	435.34***
Error B	64	15.453	2415	

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).

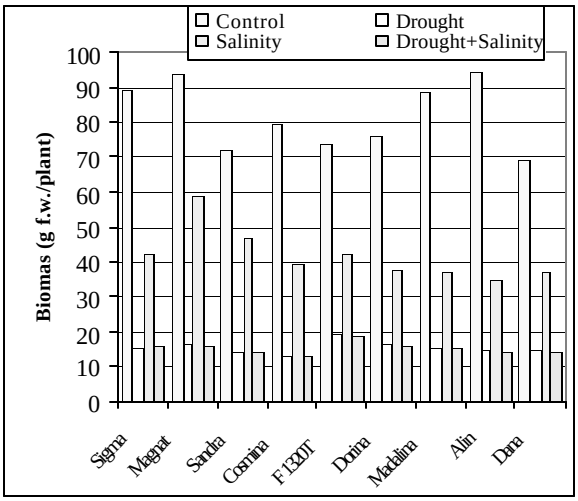

Figure. 1. The effects of stresses on biomass accumulation in studied alfalfa genotypes

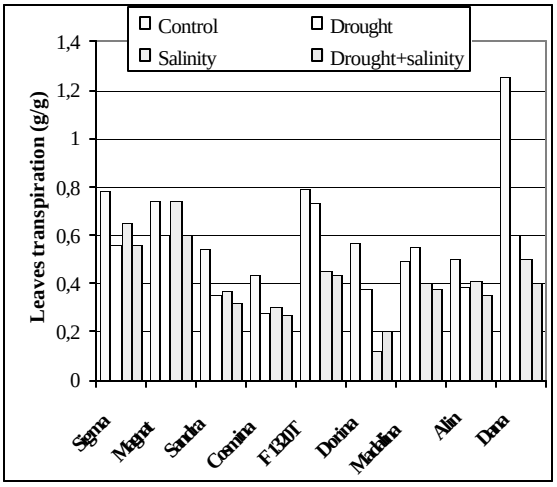

Figure 2. The effects of stresses on leaves transpiration in studied alfalfa genotypes

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 combined 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

Specification	Transpiration		
	Drought	Salt	Drought + salt
Biomass	r = 0.82* y = 0.0643x – 0.5117	r = 0.76* y = 0.0166x – 0.2402	r = 0.35 y = 0.0276x – 0,0328
	Proline content		
	r = -0.64* y = -3.3x + 103.44	r = 0.088 y = 1.1864x + 241.12	r = -0.64* y = -29.001x + 647.8

The negative effect of salinity and combined stress on alfalfa growth could be attributed to an osmotic effect. 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.

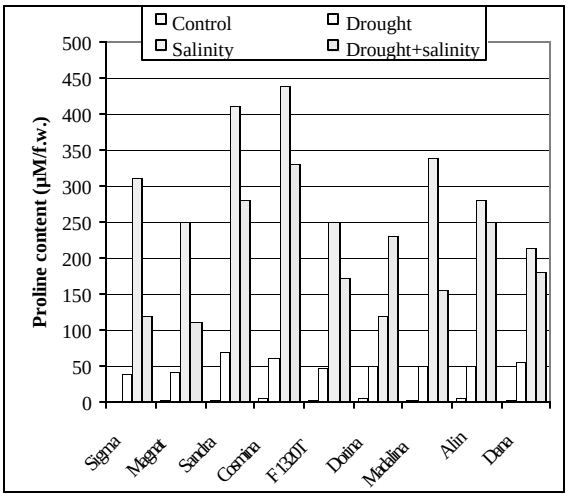

Figure 3. The effects of stresses on proline content in studied alfalfa genotypes

The significance of proline accumulation in osmotic adjustment is still debated and varies according 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 closing stomata (to decrease the transpiration) and from increasing energy and carbohydrate use in osmoregulation.

The leaves transpiration and biomass accumulation were correlated, suggesting that the former 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 earlier stages of vegetation, when alfalfa is very sensitive to this kind of stresses.

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