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THE EFFECT OF WATER STRESS ON STOMATAL RESISTANCE AND CHLOROPHYLL FLUORESCENCE AND THEIR ASSOCIATION WITH ALFALFA YIELD

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ABSTRACT

Drought stress is one of the main environmental factors limiting plant photosynthesis. Due to climate change drought problems in the world's agriculture will increase.

Alfalfa, drought-tolerant crop, generally tolerates water shortages quite successfully, but its yield may fluctuate appreciably under drought conditions. Therefore, strategies for sustainable use of water and drought resistance improvement based on the physiological traits are urgent and physiological approaches should be integrated in conventional breeding. The first condition for a successful introduction of physiological traits in a breeding program is to determine the degree of genetic variability for the trait (traits) of interest.

Research was performed with 24 alfalfa genotypes under vegetation house conditions at two water status regimes. The objective was to identify the available genetic variation and to establish efficient testing methods for characters which might positively influence alfalfa performance under drought conditions.

Among the many characters that might be important for alfalfa performance under water stress, our research focused on stomatal resistance and photochemical PSII chlorophyll fluorescence parameter (Fv/Fm, index of performance).

The analysis of variance regarding stomatal resistance showed a very significant influence of the treatment, genotype and their interaction. The variance of treatment was higher than the variance due to genotypes.

The quantum of photosynthetic yield efficiency (Fv/Fm) was not significantly influenced by drought stress but the index of performance increased very significantly in drought stressed plants, compared to the control.

There were significant correlations between stomatal resistance and biomass yield and also between stomatal resistance and drought susceptibility index, which opens new opportunities for improving drought resistance in alfalfa. We suggest that for improving resistance of alfalfa synthetic cultivars to drought, each of the genotypes components must have at least some of the traits involved in drought resistance.

Key words: water stress, stomatal resistance, chlorophyll fluorescence, drought susceptibility index.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the main forage legume in the crop livestock systems of Romania and many European countries, and it produces the greatest amount of forage protein per unit area among the legumes (Schitea, 2010). Although alfalfa is one of the most drought resistant crops, breeding for drought resistance is necessary to ensure a more complete expression of the genetic yielding potential in the conditions of predicted climatic changes (Petcu et al., 2009). This more so as the tetrasomic inheritance (2n=4x=32) in autotetraploid alfalfa and the pronounced inbreeding depression (Flajoulot et al., 2005), have caused the genetic improvement of alfalfa to be less than other major crops.

The main effects of drought stress on shoot water status are the decrease in leaf water potential, photosynthesis and growth, due to stomatal closure and lower CO_2 supply to the carboxylating enzymes (Radin et al., 1994). Stomatal control and root hydraulic conductivity or variations in root morphology are considered the key controlling factors for WUE under drought (Sharp et al., 2004).

Many studies found the chlorophyll *a* fluorescence as a very sensitive probe of physiological status of leaves and plant performance in a wide range of situations (Baker and Rosenqvist, 2004). The aim of this work was to identify the available genetic

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variation and to establish efficient testing methods for characters which might positively influence alfalfa performance under drought conditions in order to select drought resistant genotypes as components of alfalfa synthetic cultivars.

MATERIAL AND METHODS

Twenty four alfalfa genotypes were studied. The experiments were conducted in a vegetation house using Mitcherlich pots filled with a soil-sand mixture (3:1).

The alfalfa selected plants were sown during autumn (October) and than thinned to one plant/plot after two weeks. Four replicate pots were used per cultivar and the treatments. Pots were maintained at the field capacity until middle of November when the above ground parts were harvested to ensure an adequate build-up of energy reserves for survival through the winter.

Control and drought treatments

The optimal soil volumetric water content, corresponding to 70% from field water capacity, was maintained by daily irrigation (watering). Drought treatments were imposed before flowering (45-day-old plants) by maintaining soil moisture at 40% from field water capacity. Water stress duration was 14 days.

In order to avoid nutrient differences between control and stressed plants during the drought period, 100 ml Hoangland nutrient solution was added to each pot one week before drought treatment At the nutritional level, this Hoangland nutrient solution was adequate to allow correct alfalfa growth (normal leaf colour and development) for control and drought treatments.

Analyses

Drought susceptibility index. The measures of drought susceptibility index (DSI) were calculated by using mean biomass yield.

The DSI (Fischer and Maurer, 1978) was calculated as follows:

DSI = (1-Yd/Yw)/D, where Yd = mean yield of genotype under drought, Yw = mean yield of genotype under well-watered conditions and D (environmental stress intensity) = 1 – (mean yield of all genotypes under drought/mean yield of all genotypes under well-watered conditions).

Stomatal resistance was measured on upper leaves using a Delta T prometer. The calibration curve was made each time before starting measurements.

Chlorophyll fluorescence. The quantum of photosynthetic yield efficiency (Fv/Fm) and index of performance were measured using a direct fluorometer (Handy PEA, Hansatech Instruments, Kings Lynn, UK). The leaves were kept 20 minutes in the dark under the laboratory conditions. All fluorescence measurements were taken in the middle part of the plant, in fully expanded leaves, with three replicates for each treatment. Measurements of minimal (F0) and maximal (Fm) fluorescence yields allowed determination of the optimal quantum yield (Fv/Fm), the ratio (Fm – F0)/Fm. The Performance Index (PI) is one of the Chl fluorescence parameters that provide useful and quantitative information about the state of plants and their vitality (Strasser et al., 2000). The PI is a combined measure of three partial performances, namely those related to the amount of photosynthetic reaction centres, the maximal energy flux that reaches the PSII reaction centre, and the electron transport at the onset of illumination.

RESULTS

Fodder yield and drought susceptibility index

Plant growth, expressed as biomass accumulation was lower under water stress than in control. Highest yields were obtained in cultivars F 1615-04, F 1914-07, F 1918-07 and F 2017-08 under optimal conditions and obviously the fodder yield was higher in the second cutting for all genotypes.

In the first cutting under water stress, the fodder yields were higher (27.96 average g/pl and up to 40 g, in cultivar F 1535) compared with second cutting, when, the yield did not exceed 29 g fresh matter (F 1412-02 and F 2017-08) and was on average 22.38 g/pl). DSI values for the yield ranged from 0.66 to 1.26. Eleven cultivars (including the cultivars: Daniela, Roxana, F 1543, F 1608) were found to be resistant (DSI values <1), while the other 13 varieties were relatively susceptible to drought (DSI>1), (Table 1). Genotypes with low DSI values (less than 1) can be considered drought resistant (Bruckner and Frohberg, 1987), because they exhibited smaller yield reductions under water stress compared with well-watered conditions than the mean of all genotypes.

Table 1. Fodder yield of studied alfalfa cultivars and drought susceptibility index

Genotype	First cutting (g/pl)		Second cutting (g/pl)		Average (g/pl)		Drought susceptibility index
	Control	Water stress	Control	Water stress	Control	Water stress	DSI
Magnat	51	20	63	20	57	20	1.22
Daniela	43	36	47	22	45	29	0.67
Roxana	45	35	55	24	50	29.5	0.77
F 1412-02	41	37	76	29	58.5	33	0.82
F 1413-02	42	31	63	19	52.5	25	0.99
F 1414-02	32	30	53	20	42.5	25	0.78
F 1543-03	40	38	52	22	46	30	0.66
F 1535-03	48	40	60	21	54	30.5	0.82
F 1608-04	40	31	52	24	46	27.5	0.76
F 1610-04	50	25	56	22	53	23.5	1.05
F 1615-04	49	29	74	23	61.5	26	1.09
F 1710-04	43	23	69	26	56	24.5	1.06
F 1711-05	51	14	55	21	53	17.5	1.26
F 1712-04	40	38	63	17	51.5	27.5	0.88
F 1715-05	37	32	55	20	46	26	0.82
F 1814-07	45	25	66	21	55.5	23	1.10
F 1821-06	46	26	61	23	53.5	24.5	1.02
F 1913-07	38	25	55	24	46.5	24.5	0.89
F 1914-07	55	26	64	25	59.5	25.5	1.08
F 1916 -07	46	17	58	21	52	19	1.20
F 1918-07	55	23	83	19	69	21	1.31
F 2007-08	52	25	63	19	57.5	22	1.16
F 2010-08	61	27	56	26	58.5	26.5	1.03
F 2017-08	58	18	67	29	62.5	23.5	1.18
Average	*46.17*	*27.96*	*61.08*	*22.38*	*53.63*	*25.17*	

Stomatal resistance

The analysis of variance regarding stomatal resistance showed a very significant influence of the treatment, genotype and their interaction. The variance of treatments was higher than the variance due to genotypes (Table 2).

Table 2. Analysis of variance for stomatal resistance

Source of variation		Stomatal resistance		
	DF	Sum of squares	Mean square	F value
Treatment	1	28574.82	28574.82	17596.61***
Error A	2	3.2774	1.6237	
Genotype	23	3450.543	150.0236	34.5527***
Interaction	23	2940.73	127.8578	29.4476***
Error B	92	399.4531	4.3419	

*** significant differences at 0.001 probability level

Stomatal resistance of alfalfa genotypes studied ranged from 12 to 40 s/cm under water stress conditions, with an average of 24.31 for "*resistant*" genotypes and 33.82 for "*susceptible*" ones. Generally the stomatal resistance was higher for *susceptible* genotypes than for resistant ones, but there were some *susceptible* genotypes with low stomatal resistance and conversely. This could open new opportunities to improve drought resistance (Figure 1).

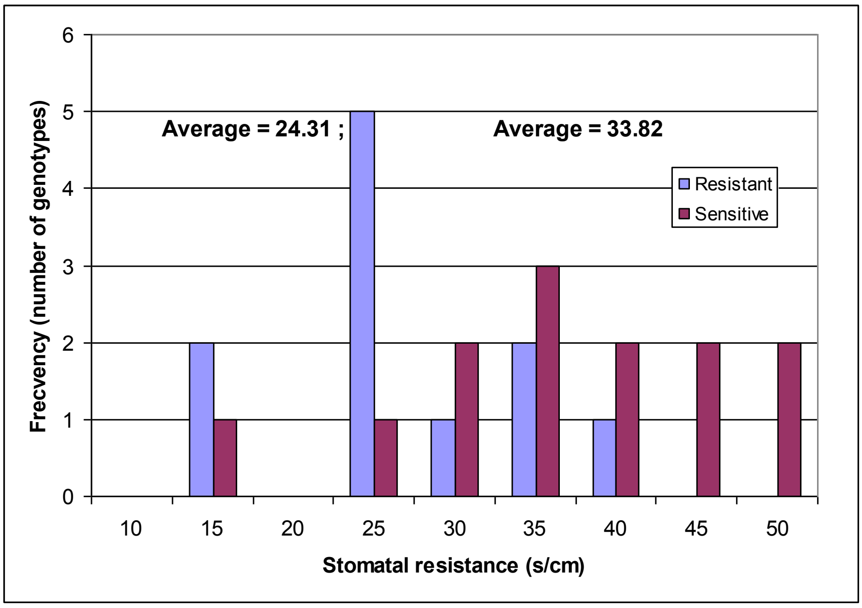

Figure 1. Frequency distribution for stomatal resistance in studied alfalfa genotypes with different drought resistance

Stomatal resistance (stomatal closure) and leaf growth inhibitions are among the earliest responses to drought and protect plants from extensive water loss, which might result in cell dehydration and death (Reynold-Henne et al., 2010). In very few cases genetic variability for stomatal traits has been demonstrated within the same species, although affected traits were mostly related to stomatal size and density (Bacelar et al., 2007). In our case, genetic variability existed for stomatal resistance/conductance: most of resistant genotypes had less stomatal resistance (this means higher stomatal conductance), which confirms hypothesis that higher-stomatal conductance species have better adaptation to drought. Moreover, the existence of susceptible genotypes with lower stomatal resistance and vice versa shows the complexity of genetic control of stomatal properties and opens new perspectives for breeders. We consider that the concept is especially important to agriculture, as water availability often limits production, and stomatal traits are major determinants of water use efficiency.

Identification of genetic variability for stomatal properties would provide a new tool for plant breeders to improve crop adaptation to stressful environments. This is even more for improved varieties of alfalfa synthetic cultivars, that usually are obtained through three or four generations of open pollinated reproduction of polycross seeds of selected parents.

Chlorophyll fluorescence

Chlorophyll fluorescence in vivo can provide information on the use and energy dissipation in PS II photosynthetic system. Over recent years, many researchers have published articles on this issue by analyzing the relationship between chlorophyll fluorescence and photosystem II efficiency at different plants and stress conditions. Our results show that the differences between treatments concerning chlorophyll fluorescence were not significant for ratio Fv/Fm and very significant for performance index (Table 3).

Table 3. Analysis of variance for Fv / Fm and PI

Source of variance	DF	Fv / Fm			PI		
		Sum of squares	Mean square	F value	Sum of squares	Mean square	F value
Treatment	1	4376.61	4373.61	0.99NS	52.72	52.72	525.52***
Error A	2	8812.49	4406.24		0.2007	0.1003	
Genotype	23	101234.1	4401.01	0.99 NS	23.004	1.0002	2.14 NS
Interaction	23	101292.3	4404.01	1.01 NS	19.05	0.8283	1.77*
Error B	92	405018.8	4402.37		42.87	0.4661	

*** and NS show significant differences at 0.001 probability level and no significant, respectively.

Many studies have used this ratio as an indicator for stress tolerance or susceptibility (Cavender-Bares and Bazzaz, 2004; Hermans et al., 2003). However, our studies showed that this parameter was quite insensitive to change produced by water stress on studied alfalfa genotypes. It is possible that level of water stress was not strong enough to exert a selection pressure for this trait.

Performance index in the 24 alfalfa genotypes which were characterized for this trait varied from 1.36 to 3.72 under water stress conditions, with an average of 2.34 for "*resistant*" genotypes and 2.94 for "*susceptible*" ones. There were resistant alfalfa genotypes with low performance index, but also some of them had medium or high performance index (Figure 2).

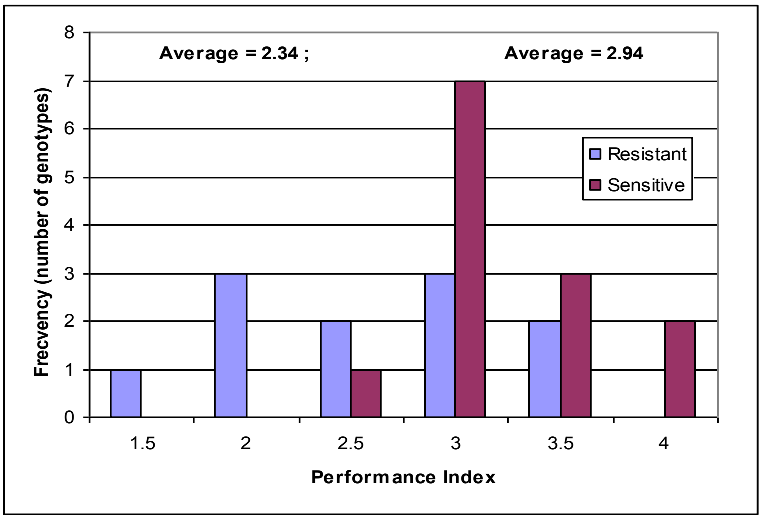

Figure 2. Frequency distribution for performance index in studied alfalfa genotypes with different drought resistance

According to the definitions of Strasser et al. (2004), the PI combines three values quantifying the three functional steps of photosynthetic activity by a PSII RC complex, from light energy absorption, trapping of excitation energy, and conversion of this energy to electron transport occurring in PSII.

The relationship between the assessed traits

The correlation among physiological traits was determined under drought stress conditions (Table 4).

The results showed that DSI had the highest positive and significant correlation with SR (r=0.59***) under drought stress. There are several reports in the literature that underlined the significant relationship between the ability to maintain low stomatal resistance and drought tolerance in several plants.

Table 4. The correlation coefficients between assessed traits in alfalfa genotypes under drought stress condition

	DSI	SR	Fv/Fv	PI
DSI	1			
SR	0.59***	1		
Fv/Fv	0.31	0.28	1	
PI	0.56***	0.57**	0.01	1

Our research showed that PI was more sensitive to drought and correlated well with plant biomass (r=0.56***) and stomatal resistance (r=0.57***).

Also, there were positive and significant correlations between Sr and PI (r=0.57***). There have been many reports showing that decreased water uptake closes stomata, which reduces photosintesis and causes an increase in chlorophyll fluorescence (Strasser et al., 2004).

Dong et al. (2008) in wheat and Yusuf et al. (2010) in alfalfa reported that under stress conditions, higher leaf water retention was a mechanism of resistance to drought, which results in a reduction of stomatal conductance and transpiration rate.

CONCLUSIONS

The results obtained from the present study showed that drought stress condition induced significant changes in all analysed physiological traits.

The loss of energy by emission of fluorescence was very significantly affected by water stress, but the differences between studied genotypes were statistically insignificant for some indicators. It is possible that the level of stress induced was not strong enough to exert selection pressure for this trait.There was significant genetic variability for stomatal resistance of alfalfa genotypes studied.

Most alfalfa genotypes with high stomatal resistance were more susceptible to water deficit. Those genotypes have a small amount of carbon dioxide, which leads to a decline in photosynthesis and therefore there is a more rapid deterioration of forage production and quality resulting from stress-induced senescence in leaves and stems.

The findings of our study showed that the assessed physiological traits could be effective for screening of drought tolerant genotypes. SR and PI could be chosen, along with biomass, as criteria for predicting drought tolerant genotypes.

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