

THE EFFECT OF WATER STRESS ON CUTICULAR TRANSPIRATION AND ITS ASSOCIATION WITH ALFALFA YIELD

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ABSTRACT

Drought effects on the water loss by cuticular transpiration and alfalfa yield of twenty four alfalfa genotypes were evaluated. The experiment was conducted under vegetation house in 2006-2007 using special plots. Water stress was imposed before flowering by restricting watering, for 14 days. Exposure of plants to drought led to noticeable decreases in both leaf water loss by cuticle and biomass accumulation.

There were significant negative correlations between water loss by cuticular transpiration and yield under water stress conditions for first and second year of alfalfa vegetation ($r = -0.64^{***}$, $r = -0.62^{***}$), suggesting that low cuticular water loss could be used in selecting drought tolerant genotypes.

Key words: cuticular transpiration, water stress, alfalfa yield.

INTRODUCTION

Drought is generally accepted to be the most widespread abiotic stress experienced by crops, and is becoming an increasingly severe problem in many regions of the world. Romania is expectating a gradual decline in rainfall and an increase in aridity. The more drought-prone Bărăgan region of South-east Romania has experienced a gradual decline in annual rainfall during last 5 years of about 90 mm, accompanied by an increase in the number of tropical days (when maximum temperatures exceed 30°C) from 10 to over 30 during the same period (data from weather, Meteo station Fundulea).

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 predicable climatic changes.

Drought resistance can be improved by increasing the water use efficiency, and one way to achieve this is by selecting genotypes with low cuticular transpiration. Cuticular (or residual) transpiration represents the main way of water loss during night under optimal conditions and during noon under drought conditions, when stomata are closed. It was used as

selection trait in wheat breeding for drought resistance (Clarke et al., 1991; Balotă, 1995; Petcu, 2005).

Selection for drought resistance of alfalfa has been applied during last years at NARDI Fundulea, beside selection for fodder yield, seed yield, disease resistance and regrowth after cutting, to develop new synthetic cultivars (by crossing Romanian cultivars with foreign germplasm) with improved performance under drought.

The objective of the present work was to investigate cuticular transpiration and association between this trait and biomass yield under normal and water stress conditions in alfalfa 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 seeds were sown during autumn (October) and then thinned to one plant/plot after two weeks. Pots were maintained at field capacity until middle of November when the above ground parts were harvested to ensure an adequate buildup of energy reserves for survival through the winter. In the next two years two experimental treatments were imposed: control (optimal watering) and imposed water stress (the watering was stopped) before flowering for 14 days.

The fresh weight of the above ground parts was measured.

Cuticular transpiration, according to Clarke (1991) method, was measured on trifoliate leaves (five for each replicate). Following the initial weight determination, the leaves were maintained in the darkness for stomata closure under ambient room conditions, weighed again after 24 h and then dried at

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90°C. Water loss was expressed in grams of water lost per gram of leaf dry matter, using the formula:

$$\frac{[(Gi-Su)-(G24-Su)]}{Su}$$

where Gi is initial fresh weight, G24 – fresh weight after 24 h, Su is the dry weight.

RESULTS AND DISCUSSION

The growth of plants, as measured by fresh weight accumulation was lower under water stress than in control, in both experimental years. Highest yields were obtained in

cultivars Mădălina, Alina, Sandra, D 17-05, Dorinela and D 9-05 under optimal conditions.

Under less favourable conditions, 14 days without water, the yields were modest. In the first year of vegetation, in these conditions, the yield did not exceed 25.67 g fresh matter (in cultivar Sigma). In the second year, the yields were higher (up to 89.48 g), although representing only 11.9% (in cultivar D 17-05) to 50.3% (in cultivar Cosmina) from the fresh matter yield of the check (no stress) (Table 1).

Table 1. Fodder yield of studied alfalfa cultivars, average of first harvesting in the period 2006-2007

Genotip	2006		2007		Average		
	Control	Water stress	Control	Water stress	Control	Water stress	% from control
Cosmina	79.85	22.97	177.72	89.48	128.785	112.45	87.32
Magnat	93.40	16.70	175.59	84.40	134.495	101.10	75.17
Sigma	89.28	25.67	188.00	72.00	138.640	97.67	70.45
F 1320T	73.68	25.45	185.60	67.43	129.640	92.88	71.64
D 14-05	49.41	10.33	224.61	73.94	137.010	84.27	61.51
Mădălina	79.48	15.77	255.36	67.11	167.420	82.88	49.50
HS 3-05	74.09	15.28	187.18	67.08	130.635	82.36	63.05
HS 5-05	78.45	10.94	199.17	71.13	138.810	82.07	59.12
Sandra	63.11	17.78	255.99	64.10	159.550	81.88	51.32
Daniela	69.11	19.25	193.19	62.39	131.150	81.64	62.25
Dorinela	69.15	16.44	230.32	65.02	149.735	81.46	54.40
D 15-05	76.27	19.25	199.76	60.79	138.015	80.04	57.99
D 13-05	76.47	20.83	171.70	59.05	124.085	79.88	64.38
HS 6-05	62.37	12.88	231.67	65.85	147.020	78.73	53.55
D 9-05	83.25	8.34	204.92	68.48	144.085	76.82	53.32
HS 7-05	69.55	11.14	157.48	65.60	113.515	76.74	67.60
D 18-05	72.26	17.40	191.80	57.68	132.030	75.08	56.87
Alina	94.10	24.88	232.81	45.20	163.455	70.08	42.87
HS 2-05	59.11	7.55	138.65	61.87	98.880	69.42	70.21
D 16-05	56.85	13.22	136.39	54.21	96.620	67.43	69.79
LC 10-05	73.16	13.87	120.30	49.66	96.730	63.53	65.68
LC 14-05	65.20	13.47	163.03	48.00	114.115	61.47	53.87
HS 4-05	79.21	9.13	118.60	35.53	98.905	44.66	45.15
D 17-05	81.95	15.66	232.23	27.69	157.090	43.35	27.60

The analysis of variance regarding water loss by cuticular transpiration shows a very significant influence of the treatment, genotype and their interaction. The variance of treatment was higher than the variance due to genotypes (Table 2).

The quantity of water lost through cuticle is generally up to 10-20 time lower than water loss by stomata. Nevertheless, for plants under

water stress conditions, when the stomata are closed, it represents the main way of water loss.

Table 3 shows the water loss by cuticular transpiration for the tested genotypes under normal watering and water stress conditions in the first and second year of vegetation. The decrease of water loss under water stress conditions is obvious.

ELENA PETCU ET AL.: THE EFFECT OF WATER STRESS ON CUTICULAR
TRANSPIRATION AND ITS ASSOCIATION WITH ALFALFA YIELD

Table 2. Analysis of variance for cuticular transpiration
(data from 2006)

Source of variation	DF	Cuticular transpiration		
		Sum of squares	Mean square	F value
Treatment	1	0.3350	0.3354	118.19***
Error A	2	0.0057	0.0028	
Genotype	23	1.3053	0.0568	54.97***
Interaction	23	0.9477	0.0412	39.91***
Error B	92	0.9500	0.0010	

*** significant at < 0.001 level of probability

Howere, cultivars reacted differently to water stress. For example, genotype Cosmina, which had the highest cuticular transpiration under no stress, had one of the lowest cuticular transpiration under water stress. On the other hand, genotypes Magnat, Sanda or Dorinela had relatively low cuticular transpiration under both conditions, while the genotypes HS 5-05, D 13-05 or D 15-05, had the highest water loss

through cuticle, in both growing conditions and experimental years. The correlation between cuticular transpiration of not stressed leaves and cuticular transpiration of stressed leaves of all studied cultivars was not significant, $r = 0.29$ (Figure 1).

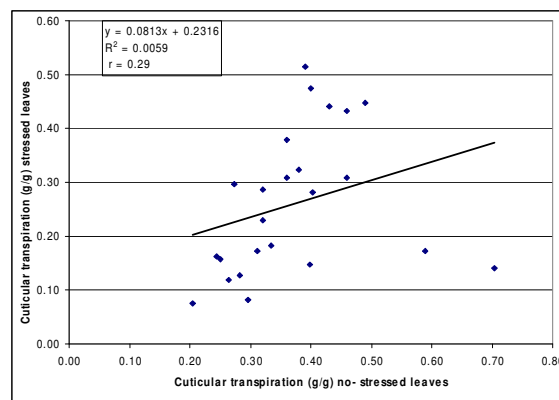


Figure 1. The effect of water stress on cuticular transpiration in twenty four alfalfa genotypes

Table 3. Cuticular transpirations (g water loss/g dry matter) for alfalfa genotypes for control and water stress conditions in 2006 and 2007

Genotype	2006		2007		Average	
	Control	Water stress	Control	Water stress	Control	Water stress
Magnat	0.24	0.16	2.41	0.50	1.33	0.33
Sandra	0.28	0.13	1.71	0.78	0.99	0.45
Cosmina	0.70	0.14	1.92	0.86	1.31	0.50
D 16-05	0.43	0.44	2.58	0.88	1.51	0.66
Dorinela	0.33	0.18	1.63	0.88	0.98	0.53
LC 10-05	0.31	0.17	2.18	0.91	1.25	0.54
Mădălina	0.32	0.29	1.73	1.00	1.02	0.64
Daniela	0.26	0.12	1.80	1.00	1.03	0.56
D 9-05	0.49	0.45	1.71	1.06	1.10	0.75
F 1320T	0.30	0.08	2.26	1.17	1.28	0.62
HS 3	0.32	0.23	1.74	1.20	1.03	0.71
D 14-05	0.36	0.38	1.79	1.22	1.07	0.80
Alin	0.40	0.15	2.00	1.23	1.20	0.69
HS 2	0.38	0.32	1.54	1.29	0.96	0.80
D 15-05	0.40	0.47	1.36	1.29	0.88	0.88
D 13-05	0.46	0.43	1.68	1.33	1.07	0.88
Sigma	0.20	0.08	2.23	1.33	1.22	0.70
HS 6	0.27	0.30	1.38	1.36	0.82	0.83
D 18-05	0.59	0.17	1.29	1.67	0.94	0.92
HS 5-05	0.39	0.51	1.89	1.69	1.14	1.10
HS 7-05	0.25	0.16	1.83	1.89	1.04	1.02
HS 4-05	0.36	0.31	2.08	2.00	1.22	1.15
LC 14-05	0.40	0.28	2.13	2.13	1.26	1.20
D 17-05	0.46	0.31	2.11	2.33	1.29	1.32

There were significant negative correlations between water loss by cuticular transpiration and fresh matter under water stress conditions for first year of vegetation ($r = -0.64^{***}$, Figure 2) and for second year of vegetation ($r = -0.62^{***}$, Figure 3), suggesting that low cuticular water loss could be used in selecting drought tolerant cultivars.

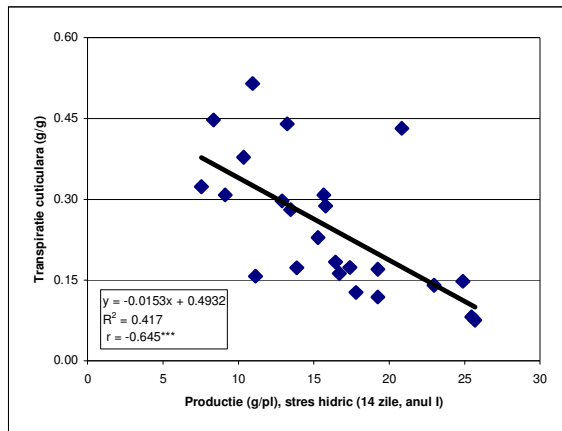


Figure 2. Relationship between yield (fresh matter) and cuticular transpiration under water stress in first year of vegetation

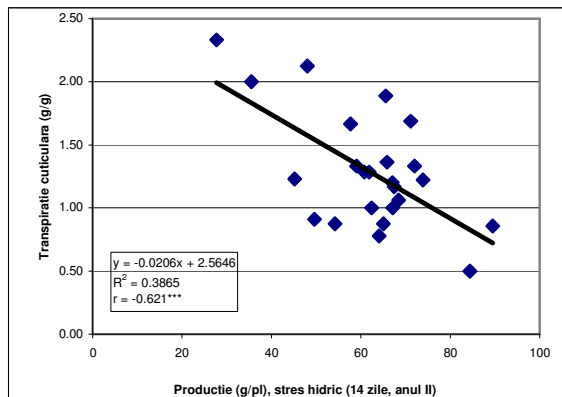


Figure 3. Relationship between yield and cuticular transpiration under water stress in second year of vegetation

Similar results were obtained in wheat ($r = -0.602^*$), (Petcu, 2005). Variation of cuticular transpiration is generally attributed

to differences in glaucousness (Fischer and Wood, 1979).

Glaucousness would be advantageous in reducing water loss through the cuticle. Indeed, Blum (1982) suggested that permeability of the cuticle to water is affected by glaucousness.

CONCLUSIONS

The results of this study indicate a strong negative effect of water stress on cuticular transpiration and yield of studied alfalfa genotypes.

The significant negative correlation between cuticular transpiration and yield suggests that cuticular transpiration is an important factor explaining the genotypic differences in drought resistance among the studied alfalfa genotypes.

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