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DROUGHT STRESS RESPONSES OF ALFALFA (*MEDICAGO SATIVA* L.) BREEDING POPULATIONS

Marijana Tucak*, Svetislav Popovic, Tihomir Cupic, Goran Krizmanic

Agricultural Institute Osijek, Department of Forage Crops Breeding and Genetics,
Juzno predgradje 17, 31000 Osijek, Croatia

*Corresponding author. E-mail: mtucak@poljinos.hr

ABSTRACT

The objectives of this research were to determine the effect of drought stress on yield in 23 alfalfa breeding populations and one cultivar, to evaluate the relationships between long-term alfalfa yields in second and third year of production and total precipitation during the alfalfa growing season, to screen and select breeding populations with high yield potential in drought conditions. The study was conducted in two consecutive years (2011 and 2012) at the experimental field of the Agricultural Institute Osijek, Croatia. The field trial was arranged in a randomized complete block design with four replications. Populations and the cultivar were planted in plots with size of 7.2 m^2 in sowing rate of 15 kg ha^{-1}. The yields of green mass and dry matter were determined in the second and third growing seasons, where in each year of the investigation experiment was cut off five times. Breeding populations ABP 13 obtained the highest average yield of green mass of dry matter during two consecutive dry years. Populations ABP 8, 3, 11 and 17 had high yields. It was confirmed that alfalfa has high ability to tolerate drought by evaluating the relationships between long-term yields of established alfalfa and total precipitation during the growing season. Populations ABP 13, 8, 3, 11 and 17 showed high yield potential and stability under drought stress and presented valuable genetic resources for our future breeding work.

Key words: alfalfa, climate change, drought, forage yield, screening and selection.

INTRODUCTION

The impact of global climate change on crop production is one of the main priorities for research over the past decade. Environmental stresses are limiting factors of production of important agricultural crops species worldwide (Gaspar et al., 2002; Roy et al., 2011; Shanker et al., 2014; Nezhadahmadi et al., 2015; Inostroza et al., 2015; Mitrica et al., 2015). The most important abiotic stress is drought that can limit the growth, development and productivity of many crops. The reaction of plant to stress depends on the duration, severity and frequency of exposure to stress and their mutual interaction in the environment. Yield losses caused by drought are different in different crops, depending on the development stage of the crop during stressful conditions and the plant species. They can range from 14 to 50% (Baric et al., 2008; Kobraee and Shamsi, 2012; Khokhar and Teixeira da Silva, 2012; Harrioson et al., 2014).

Alfalfa (*Medicago sativa* L.) is the most widely used and one of the most important perennial crops in the world. Beside higher yields, favourable nutritional value and numerous positive effects on the environment, this crop is characterized by a severe drought tolerance (Real et al., 2011; Lang and Vejrazka, 2012; Liatukien and Liatukas, 2014; Petcu et al., 2014). Established alfalfa has a strong, powerful and deep root system and very well can use reserves of water stored in the deeper layers of the soil below the reach of shallow-rooted grasses and cereals (Jefferson and Cutforth, 1997; Annicchiarico et al., 2013; Annicchiarico et al., 2014; Vasileva and Kostov, 2015). Numerous authors have reported the ability of alfalfa to deplete water from 3-4 m deep in the soil profile, and below the laboratory upper limit of soil water extraction (Cutforth et al., 1991; Campbell et al., 1994). Alfalfa may well survive the deficit of affordable soil moisture (water stress that can last a longer time). In such conditions, one to two of cuts can be lost, yields are lower, but

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as soon the water supply raise, alfalfa quickly recover aboveground vegetative mass. This indicates that alfalfa yield reduction appears as a result of stressful conditions caused by drought, but it is less pronounced compared to other forage crops and cereals.

The objectives of this research were: (1) to determine the effect of drought stress on yield in 23 alfalfa breeding populations and one cultivar, (2) to evaluate the relationships between long-term alfalfa yields in second and third year of production and total precipitation during the alfalfa growing season, (3) to screen and select breeding populations with high yield potential in drought conditions.

MATERIAL AND METHODS

The study was conducted in two consecutive years (2011 and 2012) in Eastern Croatia at the experimental field of the Agricultural Institute Osijek. Twenty two breeding populations of alfalfa (ABP 1-22) of different origin and one control cultivar (OS-99) created at the Department of Forage Crops Breeding and Genetics Agricultural Institute Osijek were investigated.

The experiment was sown at the end of March 2010 as randomized complete block design with four replications. Populations and the cultivar were planted in plots with size of 7.2 m^2 in sowing rate of 15 kg ha^{-1}. The experiment was not irrigated, fertilized or protected against weed/pest/diseases. The year 2010 of alfalfa establishment was not involved in this study. Measurements of yield were conducted in the second and third alfalfa growing seasons (2GS, 3GS), where in each year of the investigation, experiment was cut off five times (11/05, 07/06, 07/07, 08/08, 12/09 in 2011 and 09/05, 18/06, 19/07, 20/08, 15/10 in 2012).

In both experiments, all cuts plants were cut in the budding stage-beginning of flowering. The yield of green mass was determined directly in the field by measuring the cut stalk weight of the whole parcel of all populations and cultivar at each cut with harvester Hege 212 with electronic scales and yields calculated in t ha^{-1}. In every cut before cutting from all plots every populations/ cultivar fresh forage samples (approximately 500 g) were taken randomly from the middle row plots for determination of dry matter. After weighting, the samples were dried at 105°C for 24 h and weighted again to determine the percentage of dry matter. The dry matter yield was calculated as dry matter content × green mass yield/100. A two-way ANOVA was performed by CropStat version 7.2 statistical program and differences among means of statistically significant parameters were expressed by the LSD test (IRRI, 2009).

In both years, the average monthly air temperatures during the growing season of alfalfa were similar and did not differ significantly from the long-term averages (data not presented). Total annual rainfall during the study period (2011: 310.7 mm; 2012: 358.6 mm) were significantly less compared to the long term average (467.8 mm) (Source: Statistical Yearbook of the Republic of Croatia, Figure 1).

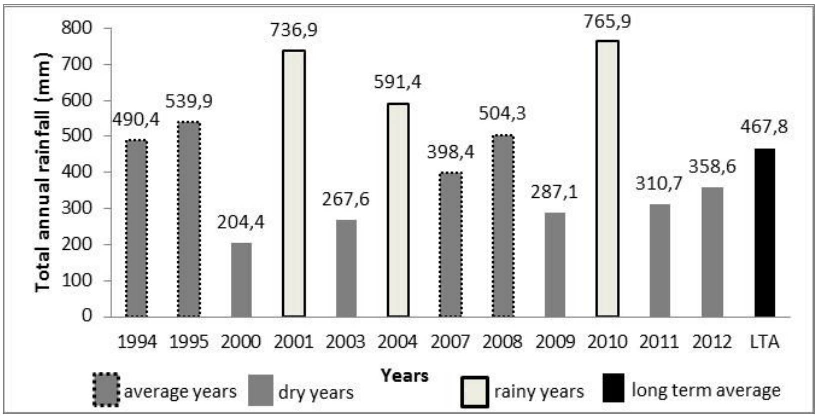

Figure 1. The total annual rainfall for the alfalfa growing season (3-10 months) during the study (2011 and 2012), long-term research on alfalfa study (1994-2010) and long term average (LTA) for Osijek (1971-2000)

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences between alfalfa breeding populations/cultivar for green mass and dry matter production in the third year and on average of two years of examination (Tables 1 and 2). Differences were significant between years for yield of green mass while the interaction year x population was not statistically significant.

Breeding population ABP 13 produced the highest average yield of green mass and dry matter in both years of investigation as well as in the two-year average (Table 2).

Yield of GMY population ABP 13 was higher by 14.52% (80.01 t ha^{-1}, in the third production year) to 20.37% (101.18 kg ha^{-1}, second production year) compared to the average annual yield of all cultivars (68.39 and 80.56 t ha^{-1}). Population ABP 13 had a higher yield of green mass in both studied growth seasons as well as on the two-year average (17%, 12.75% and 12.15%) compared with the control cultivar OS-99. Average yields of dry matter of population ABP 13 (20.29 t ha^{-1}, 18.60 t ha^{-1}, 19.44 t ha^{-1}) were higher than the values recorded in the control cultivar and average annual yields of all cultivars in both growing seasons, while the yield increase from this population ranged from 15.86% to 20.84%. High yields of green mass and dry matter had also populations ABP 8, 3, 11 and 17. The lowest values of yield in both seasons, as well as on the two-year average were observed in populations ABP 20. Annual average yield of green mass of all cultivars obtained in the second production year (80.56 t ha^{-1}) was higher than the yields achieved in the third production year. The yield differences between the observed years were expected because alfalfa is a perennial crop and the expression of genetic potential is influenced (besides population/cultivar) by age of the stand. The obtained result is in accordance with research by many authors that have shown that alfalfa maximum yield potential is achieved in the second production year (Tucak et al., 2008; Sun et al., 2011; Avci et al., 2010).

Table 1. Results of two-way ANOVA for yields of green mass and dry matter (GMY, DMY) of 23 alfalfa breeding populations/cultivar

Source of variation	Degree of freedom	Sum square		Mean square		F-value	
		GMY	DMY	GMY	DMY	GMY	DMY
Repetition	3	9119.58	365.02	3039.86	121.67	9.86 ns	10.04 ns
Year (Y)	1	6811.42	7.92	6811.42	7.92	22.10*	0.65 ns
Error year	3	924.27	36.33	308.09	12.11		
Population (P)	22	4173.50	204.59	189.70	9.29	4.67**	7.46**
Y x P	22	893.23	27.39	40.60	1.24	0.51 ns	0.36 ns
Error	132	10445.84	448.48	79.13	3.39		
Total	183	32367.86	1089.76	176.87	5.95		

*Significant at $p<0.05$; **Significant at $p<0.01$, ns – not significant.

In the both years of study during the growing season significantly less rainfall compared to the long term average was observed, which gave indication of dry years. Stressful conditions caused by drought in two consecutive years resulted in reduced yields in most of the populations of alfalfa. The losses in the yield of the conducted research as compared with the yields achieved in years with favorable distribution of precipitation are significantly smaller, or in some populations insignificant, compared to losses in most other crops. This was confirmed by analysis of the relationships between the total annual rainfall during the alfalfa growing season and average annual yield of green mass and dry matter of alfalfa in the second and third seasons growth obtained in studies of different breeding populations/cultivars/ecotypes in a series of comparative experiments at Agricultural Institute Osijek in the period since 1994 to 2010 (Figures 1, 2 and 3).

Table 2. Average yields of green mass and dry matter of 23 alfalfa breeding populations/cultivar in second and third growth seasons (2011 and 2012), Agricultural Institute Osijek

Populations/ cultivar	Grean mass yield (t ha^{-1})			Dry matter yield (t ha^{-1})		
	2011	2012	Average	2011	2012	Average
ABP 1	78.20	67.88	73.04	16.39	15.73	16.06
ABP 2	73.47	65.94	69.71	15.12	15.55	15.33
ABP 3	85.02	70.10	77.56	17.16	16.43	16.79
ABP 4	81.14	68.21	74.67	16.46	15.82	16.14
ABP 5	81.55	67.81	74.68	16.63	15.08	15.86
ABP 6	84.60	70.08	77.34	16.90	16.01	16.45
ABP 7	81.70	65.25	73.48	16.03	15.13	15.58
ABP 8	86.08	71.92	79.00	16.73	16.93	16.83
ABP 9	81.15	67.28	74.22	15.68	15.55	15.61
ABP 10	74.20	64.70	69.45	15.25	14.88	15.07
ABP 11	84.74	70.48	77.61	16.74	16.16	16.45
ABP 12	80.49	68.15	74.32	16.00	15.96	15.98
ABP 13	101.18	80.01	90.59	20.29	18.60	19.44
ABP 14	74.32	68.86	71.59	15.42	15.58	15.50
ABP 15	83.74	64.70	74.22	16.72	14.90	15.81
ABP 16	74.57	67.82	71.20	15.32	15.52	15.42
ABP 17	84.47	71.33	77.90	16.56	16.28	16.42
ABP 18	76.69	68.14	72.41	14.98	15.73	15.35
ABP 19	74.01	64.56	69.28	14.00	14.04	14.02
ABP 20	66.16	62.02	64.09	13.30	13.92	13.61
ABP 21	78.45	68.30	73.37	15.37	15.15	15.26
ABP 22	82.98	69.69	76.34	15.89	15.55	15.72
OS-99	83.97	69.80	76.89	16.52	15.42	15.97
Average	80.56	68.39	74.48	16.06	15.65	15.85
LSD 0.01		8.31	11.62		2.19	2.40
LSD 0.05		6.25	8.79		1.65	1.82
LSD 0.05 year	8.23					

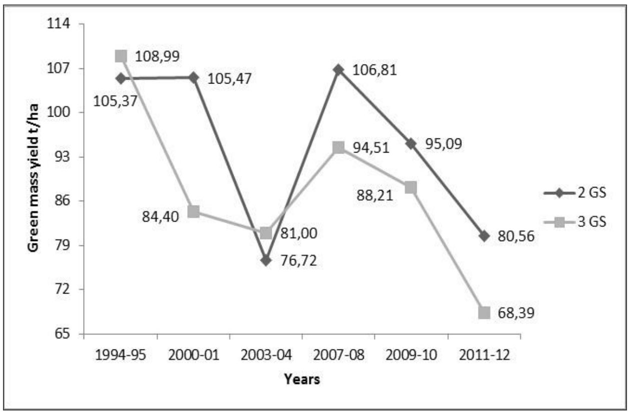

Figure 2. The average annual yield of green mass of alfalfa in the second and third growth seasons (2GS, 3GS) obtained in studies of different breeding opulations/cultivars/ecotypes in a series of comparative experiments at Agricultural Institute Osijek

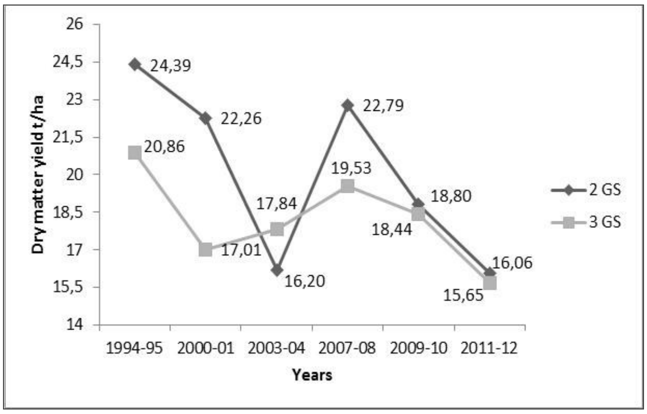

Figure 3. Average annual dry matter yield of alfalfa in the second and third growth seasons (2GS, 3GS) obtained in studies of different breeding populations/cultivars/ecotypes in a series of comparative experiments at Agricultural Institute Osijek

In dry years (2000, 2003, 2009, 2011, 2012) the average annual yield of green mass of alfalfa ranged from 68.39 t ha^{-1} (3GS) to 105.47 t ha^{-1} (2GS) and dry matter yield varied from 15.65 t ha^{-1} (3GS) up to 22.26 t ha^{-1} (2GS) (Figures 2 and 3). Yields in drought years were significantly lower (from 94.51 to 108.99 t ha^{-1} for GMY the 3GS, from 19.53 t ha^{-1} 3GS to 24.39 t ha^{-1} 2GS for DMY) in years with normal rainfall (1994, 1995, 2007, 2008) compared with average annual yields except in 2012 (68.39 t ha^{-1}), which probably was associated with severe water deficit in the soil occurred due to extremely dry period in the previous year. In rainy years (2001, 2004, 2010), average annual yields (from 81.00 t ha^{-1} in 2004 to 88.21 t ha^{-1} in 2010 for GMY; 1.17 t ha^{-1} in 2001 to 18.44 t ha^{-1} in 2010 for DMY) were similar to or lower than the yields in years with normal or less rainfall during the alfalfa growing season, which is expected since the alfalfa was in the third season in all observed years.

The best response of alfalfa on drought stress was obtained by Lang and Vejrazka (2012) by evaluating effects of a precipitation deficit on yield and quality of different forage legumes. Real et al. (2011) tested production and persistence of a large group of promising perennial species and accessions in six Mediterranean environments. Kizekova et al. (2013) studied the impact of drought on the yield stability and quality on red clover, alfalfa, and their mixture during two consecutive extremely dry years. Peterson et al. (1992) were examining the effect of drought on the herbage yield on quality of four perennial forage legumes (birdsfoot trefoil, Cicer milkvetch, red clover, alfalfa) and found that alfalfa achieved significantly higher average herbage yield (from 120% to 160%) and favourable quality traits of all tested legumes. Listed authors concluded that although drought reduced the herbage yield of all legumes, alfalfa has the greatest yield potential during drought.

CONCLUSIONS

Breeding population ABP 13 produced the highest average yield of green mass of dry matter during two consecutive dry years. Populations ABP 8, 3, 11 and 17 had high yields. By evaluating the relationships between long-term yields of established alfalfa and total precipitation during the growing season, it was confirmed that alfalfa has high ability to tolerate drought. Populations ABP 13, 8, 3, 11 and 17 showed high yield potential and stability under drought stress and represent valuable genetic resources for future breeding work.

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