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AGRO-MORPHOLOGICAL AND FORAGE QUALITY TRAITS OF SELECTED ALFALFA POPULATIONS AND THEIR APPLICATION IN BREEDING

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ABSTRACT

The objectives of this research were to evaluate agro-morphological and forage quality performances of selected alfalfa populations and to predict the genetic potential of this populations as a source of the material for breeding programs and/or commercial use. Twenty selected alfalfa populations and two control cultivars were tested over three consecutive years (2008, 2009, 2010) at the experimental field of the Agricultural Institute Osijek in Croatia. The experimental design was a randomized complete block with three replications. The plot size was 6 m^2 at the sowing rate of 15 kg ha^{-1}. A total of fifteen traits were measured/analysed on all plots and/or individual plants of all populations and cultivars. Significant differences were determined between alfalfa populations/cultivars in all investigated traits, except for content of crude protein in dry matter. The highest three-years average yields (from 86.32 to 101.12 t ha^{-1} for green mass, from 17.45 to 20.46 t ha^{-1} for dry matter, from 3657 to 4289 kg ha^{-1} for crude protein) and favourable values for the most of the investigated morphological and forage quality traits were obtained in populations MSP 16,8,1,9,20,11,10. Seven superior alfalfa populations were identified which could represent a valuable source of genetic material for further selection and improvement of our breeding program and/or application for plant cultivar registration.

Keywords: Alfalfa, agro-morphological traits, breeding, forage quality, population

INTRODUCTION

Alfalfa is one of the most widely cultivated forage legumes, that is cut off for hay and silage and/or growth in pasture for grazing, in the world because of its high nutritional quality for livestock, high biomass production, broad adaptability to a wide range of ecological conditions and a favourable environmental impact (Herrmann et al., 2010; Bouton, 2012; Sabanci et al., 2013). Alfalfa breeding programs are mainly focused on yield increasing, enhancing forage nutritive value and improving of tolerance to abiotic/biotic stresses. Alfalfa is perennial species with highly genetic complexity at individual and population level due to its autotetraploidy and allogamy that is characterized by a high level of heterozygosity and severe inbreeding depression (Labombarda et al., 2000; Tucak et al., 2010). Currently, in most commercial alfalfa breeding programs, most used methods for the development of alfalfa cultivars is recurrent phenotypic selection, with or without progeny testing, with tendency to accumulate desirable alleles at high frequency into a population (Li and Brummer, 2012). Development of new alfalfa cultivars and/or populations is difficult and a very long-lasting process in which mainly is evaluated all the available germplasm of large number individual plants in nursery over multiple cuttings per year and multiple years. In each of several cycles of selection the superior plants have been selected and intercrossed to generate new populations with improved important agronomic traits (insects/diseases resistance, cold/drought tolerance, greater persistence, higher forage yield and nutritional value). After several cycles of selection, alfalfa breeders could increase the frequency of favorable individuals of important agronomic traits but it can be difficult to know if the original genetic diversity present in the breeding pool was maintained and/or increased/decreased.

Constantly maintenance genetic diversity and variability of alfalfa breeding pool during the selection through introductions of distances materials as a new source of genetic diversity is a prerequisite for successful breeding and developing of new cultivars/populations with high potential for yield and forage quality (Tucak et al., 2011; Živković et al., 2012).

The objectives of this research were to evaluate agro-morphological and forage quality performances of selected alfalfa populations and to predict the genetic potential of this populations as a source of the material for breeding programs and/or commercial use.

MATERIALS AND METHODS

Twenty selected alfalfa populations (MSP1 to MSP20) and two control cultivars (OS-88, OS-99) were tested over three consecutive years (2008, 2009, 2010) at the experimental field of the Agricultural Institute Osijek in Croatia. Investigated populations were developed after four cycles of phenotypic selection from breeding nurseries which contained diverse alfalfa germplasm in which superior individual plants have been selected for higher green mass yield and persistency. The field trial was established (17/03 in 2008) as randomized block design with three replications. The plot size was 6 m^2. Hand sowing was done at row distance of 20 cm at the sowing rate of 15 kg ha^{-1}. The experimental plots were cut four times in the first growing season (23/06, 30/07, 28/08, 27/10 in 2008) and five times in the second and third growing seasons (05/05, 04/06, 07/07, 13/08, 18/09 in 2009, 11/05, 12/06, 13/07, 15/08, 25/10 in 2010). At each cut in all growing seasons yields of green mass, dry matter and crude protein (GMY, DMY, CPY) per plot, height and regeneration of plants (PH, PR, average on five randomly selected plants/plots, cm) were measured. Yields were expressed as the average annual yield in investigated three-year experiments in t ha^{-1} for GMY and DMY, respectively, and in kg ha^{-1} for CPY. In second cut of the second growing season, using the ten randomly selected stems of all plots of each population/cultivar, number and length of internodes (NI and LI, mm) stem thickness (ST, mm) and width and length of central leaflet (WCL and LCL, mm) were determined. In the same cut, forage samples of 1kg were randomly taken from the middle rows from each plot of all populations/cultivars for leaf to stem ratio (LSR) and forage quality traits determination. The content of crude protein (CP) and neutral and acid detergente fiber (NDF, ADF) in dry matter were determined according to standard methods (AOAC, 1995). Relative feed value (RFV) was calculated according to equations adapted from common formulas for forages (Schroeder, 1994). All collected data were processed by analyses of variance applying the least significant differences test using the general linear model procedure of SAS software 9.1.3 (SAS Institute, 2002-2003).

RESULTS AND DISCUSSION

Significant differences were determined between alfalfa populations/cultivars in all investigated traits, except for content of crude protein in dry matter (Tables 1 and 2, Figure 1AB).

Table 1. Average values of the investigated agronomical traits of 22 alfalfa populations/ cultivars, 2008-2010, Agricultural institute Osijek

Populations/ cultivars	Investigated traits				
	*GMY (t ha^{-1})	DMY (t ha^{-1})	CPY (kg ha^{-1})	PH (cm)	PR (cm)
MSP1	92.21^{BC}	18.60^{BC}	3774^{ABC}	79.39^{A}	24.26^{ABC}
MSP2	75.60^{IJK}	15.61^{FGH}	3206^{C-G}	74.85^{B-G}	17.49^{IJ}
MSP3	75.03^{IJK}	15.85^{FGH}	3274^{C-G}	74.71^{B-G}	19.23^{F-J}
MSP4	79.90^{F-K}	16.45^{E-H}	3458^{C-G}	72.39^{F-I}	20.47^{D-H}
MSP5	81.38^{E-K}	16.59^{D-H}	3338^{C-G}	71.37^{GHI}	19.23^{F-J}
MSP6	80.34^{E-K}	16.54^{E-H}	3351^{C-G}	72.90^{E-I}	17.06^{IJ}
MSP7	83.04^{D-J}	17.18^{C-F}	3460^{C-G}	73.73^{D-I}	19.52^{F-J}
MSP8	98.71^{AB}	19.34^{AB}	4151^{AB}	78.28^{AB}	22.71^{CDE}
MSP9	90.25^{BCD}	18.43^{BCD}	3722^{ABC}	77.87^{ABC}	26.01^{AB}
MSP10	86.32^{C-G}	18.08^{B-E}	3690^{BCD}	74.93^{B-G}	23.28^{BCD}
MSP11	88.34^{C-F}	17.94^{B-E}	3707^{A-D}	76.09^{A-F}	22.03^{C-F}
MSP12	78.14^{G-K}	16.34^{E-H}	3599^{B-F}	71.60^{GHI}	17.35^{IJ}
MSP13	74.50^{JK}	15.07^{H}	2961^{G}	70.01^{HIJ}	17.21^{IJ}
MSP14	73.93^{K}	15.29^{GH}	3131^{D-G}	66.77^{J}	16.74^{J}
MSP15	76.63^{H-K}	15.22^{GH}	3060^{FG}	71.71^{GHI}	18.68^{G-J}
MSP16	101.12^{A}	20.46^{A}	4289^{A}	76.84^{A-E}	26.56^{A}
MSP17	85.18^{C-H}	17.30^{C-F}	3575^{B-F}	69.69^{IJ}	18.76^{G-J}
MSP18	83.81^{C-I}	16.89^{C-H}	3553^{C-F}	74.04^{C-H}	20.63^{D-H}
MSP19	85.80^{C-G}	17.01^{C-G}	3405^{C-G}	70.57^{HIJ}	17.91^{HIJ}
MSP20	89.06^{CDE}	17.45^{C-F}	3657^{B-E}	77.26^{A-D}	21.22^{D-G}
OS-88	83.39^{C-I}	16.93^{C-G}	3589^{B-F}	71.95^{GHI}	19.81^{E-I}
OS-99	76.40^{H-K}	15.96^{FGH}	3091^{EFG}	73.55^{D-I}	17.49^{IJ}
Average	83.59	17.02	3502	73.66	20.17
CV (%)	4.80	4.93	7.62	2.53	6.56
LSD 0.05	6.62	1.38	440.12	3.07	2.18
LSD 0.01	8.85	1.85	588.41	4.10	2.91

Values within columns followed by different letter are significantly different at the $P \leq 0.01$

*GMY-green mass yield; DMY-dry matter yield; CPY-crude protein yield; PH-plant height; PR-plant regeneration

Population MSP 16 had significantly the highest three-year average yields of green mass, dry matter and crude protein (101.12 t ha^{-1} GMY, 20.46 t ha^{-1} DMY, 4289 kg ha^{-1} CPY). This population had higher yields than control cultivars (for GMY up to 24.44 %, for DMY up to 21.99 %, for CPY up to 27.93 % in comparison to cultivar OS-99). Also this population was superior above mean values of all populations/cultivars (for GMY up to 17.33 %, for DMY up to 16.81 % and for CPY up to 18.34 %). High yields were determined for populations MSP 8,1,9,20,11,10 (Table 1).

Significant reduction in yield had population MSP 14 and 13. Dry matter yields obtained in this study were similar to results obtained by Avci et al. (2010) who examined the performances of phenotypically superior alfalfa lines. Our results were lower than the yields obtained from Annicchiarico et al. (2011) who studied the landraces and cultivars of different geographical origin across ten different agricultural environments.

Besides the different genetic yield potential of alfalfa materials included in these investigations, the yield differences were most likely associated with different environmental conditions where tests were conducted. The average plant height ranged from 66.77 cm (MSP 14) to 79.39 cm (MSP 1), which was similar to the results of Rimi et al. (2010) who obtained a range of plant heights from 65.9 cm to 75.0 cm. The fastest growth was recorded after cuts in population MSP 16 (26.56 cm), which was not significantly faster than the values determined regeneration in populations MSP 9 and 1 (Table 1). Population MSP 18 had the highest number of internodes per stem (12.10), which was not significantly higher than the values obtained in populations MSP 9,11,16,2,7,10,5 (Table 2). Length of internodes per stem ranged from 32.30 mm (MSP 4) to 44.00 mm (MSP 17). According to Annicchiarico et al. (2010) forage quality can be improved by selection for modified stem morphology (increased internode number, decreased internode length). This statement is confirmed by the results achieved by population MSP 11, which had a larger number of shorter internodes, the best leaf to stem ratio (1.011, Table 2), satisfying protein content, low content of NDF and ADF and therefore the highest relative feed value (160.26, Figure 1 AB). According to quality standards (Hay Marketing Task Force of the American forage and Grassland Council, RFV > 151) it was classified in the category of high-quality forage.

Table 2. Average values of the investigated morphological traits of 22 alfalfa populations/ cultivars in the second cut of the second growing season, 2009, Agricultural institute Osijek

Populations/	**Investigated traits**					
cultivars	***NI**	**LI (mm)**	**ST (mm)**	**WCL (mm)**	**LCL (mm)**	**LSR**
MSP1	10.90^{C-F}	42.00^{AB}	2.19^{A-D}	10.60^{E}	30.00^{BCD}	0.965^{AB}
MSP2	11.40^{ABC}	35.80^{B-F}	2.21^{A-D}	12.80^{AB}	30.40^{ABC}	0.879^{FGH}
MSP3	10.70^{C-F}	38.60^{A-F}	2.20^{A-D}	13.20^{A}	32.50^{A}	0.869^{GH}
MSP4	10.40^{EF}	32.30^{F}	1.95^{DEF}	12.00^{A-E}	31.30^{AB}	0.871^{GH}
MSP5	11.30^{A-D}	38.30^{A-F}	2.18^{A-D}	13.00^{A}	30.50^{AB}	0.898^{D-G}
MSP6	10.30^{F}	40.70^{A-D}	2.28^{AB}	12.80^{AB}	31.10^{AB}	0.894^{D-G}
MSP7	11.40^{ABC}	41.50^{ABC}	2.25^{ABC}	12.30^{A-E}	30.70^{AB}	0.890^{EFG}
MSP8	11.00^{C-F}	34.00^{DEF}	2.09^{A-E}	13.00^{A}	30.60^{AB}	0.955^{BC}
MSP9	11.90^{AB}	33.10^{EF}	2.24^{ABC}	13.00^{A}	30.50^{AB}	0.896^{D-G}
MSP10	11.30^{A-D}	39.20^{A-F}	2.05^{A-F}	12.80^{AB}	29.70^{BCD}	0.913^{B-G}
MSP11	11.90^{AB}	34.70^{C-F}	2.05^{A-F}	11.80^{A-E}	26.30^{FG}	1.011^{A}
MSP12	10.80^{C-F}	35.20^{B-F}	2.18^{A-D}	11.70^{A-E}	28.10^{C-F}	0.897^{D-G}
MSP13	11.20^{B-E}	35.30^{B-F}	2.04^{A-F}	11.10^{B-E}	26.80^{FG}	0.929^{B-F}
MSP14	11.00^{C-F}	34.40^{C-F}	2.30^{A}	11.80^{A-E}	29.30^{B-E}	0.880^{FGH}
MSP15	10.50^{DEF}	41.20^{A-D}	2.01^{B-F}	12.70^{ABC}	26.70^{FG}	0.897^{D-G}
MSP16	11.50^{ABC}	39.90^{A-E}	2.01^{B-F}	12.00^{A-E}	25.70^{GH}	0.956^{B}
MSP17	10.50^{DEF}	44.00^{A}	1.99^{C-F}	11.80^{A-E}	27.70^{D-G}	0.916^{B-G}
MSP18	12.10^{A}	34.70^{C-F}	1.86^{EF}	10.90^{DE}	27.10^{EFG}	0.938^{B-E}
MSP19	11.00^{C-F}	38.80^{A-F}	1.88^{EF}	12.40^{A-D}	29.60^{BCD}	0.945^{BCD}
MSP20	10.90^{C-F}	39.90^{A-E}	1.99^{C-F}	12.60^{A-D}	23.80^{H}	0.960^{AB}
OS-88	11.10^{B-F}	32.90^{EF}	2.04^{A-F}	11.00^{CDE}	27.20^{EFG}	0.836^{H}
OS-99	10.70^{C-F}	35.20^{B-F}	1.79^{F}	12.70^{ABC}	29.70^{BCD}	0.903^{C-G}
Average	11.08	37.35	2.08	12.18	28.87	0.914
CV (%)	3.45	8.86	6.08	6.43	3.61	2.61
LSD 0.05	0.630	5.45	0.208	1.29	1.72	0.039
LSD 0.01	0.842	7.29	0.279	1.72	2.30	0.052

Values within columns followed by different letter are significantly different at the $P \leq 0.01$
*NI-number of internodes; LI-length of internodes; ST-stem thickness; WCL-width of central leaflet; LCL-length of central leaflet ; LSR-leaf to stem ratio

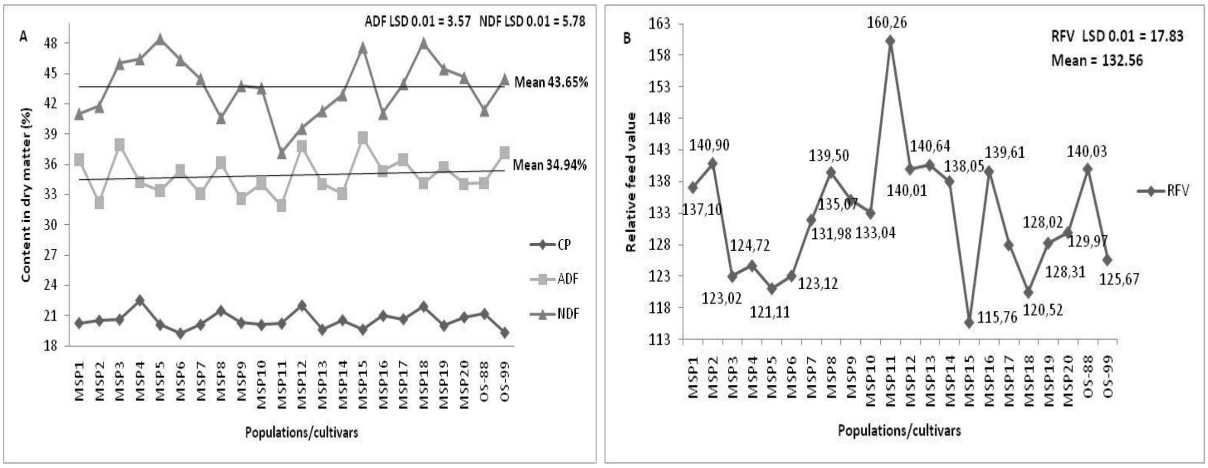

Figure 1 AB. Forage quality traits of the investigated of 22 alfalfa populations/cultivars (CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, RFV – relative feed value)

The average thickness of the stems of all populations/cultivars was 2.08 mm, and ranged from 1.79 mm (cultivar OS-99) to 2.30 mm (population MSP 14).Greatest width and length of central leaflet was found in the population MSP 3 (13.20 and 32.50 mm), while the populations MSP 20 and 1 had smallest values of these properties (Table 2). The best leaf to stem ratio had population MSP 11, which was not statistically significantly better than the values of this ratio observed in populations MSP 1 and 20. Population MSP 4 had the highest protein content (22.53 %), which was not significantly higher compared to all other observed populations/cultivars where the protein content varied from 19.27 % (MSP 16) to 22.02 % (MSP 12). Populations MSP 5 and 15 had the highest content of neutral and acidic fiber and low protein content, which resulted in lower values of RFVs of these populations (Figure 1 AB). Population MSP 11 had justifiably highest RFV value. High values of RFV were also found in populations MSP 2 and 12. The highest yields and favorable values for the most of the investigated morphological and forage quality traits were obtained at populations MSP 16,8,1,9,20,11,10. These populations represent a valuable source of genetic material for further selection and improvement of our breeding program and/or application for plant cultivar registration.

CONCLUSIONS

The highest three-years average yields (from 86.32 to 101.12 t ha^{-1} for green mass, from 17.45 to 20.46 t ha^{-1} for dry matter, from 3657 to 4289 kg ha^{-1} for crude protein) and favourable values for the most of the investigated morphological and forage quality traits were obtained in populations MSP 16,8,1,9,20,11,10. Seven superior alfalfa populations were identified which could represent a valuable source of genetic material for further selection and improvement of our breeding program and/or application for plant cultivar registration.

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