

Asian Journal of Crop Science

ISSN 1994-7879





Asian Journal of Crop Science 3 (2): 68-76, 2011 ISSN 1994-7879 / DOI: 10.3923/ajcs.2011.68.76 © 2011 Asian Network for Scientific Information

Effect of Selection for Crown Diameter on Forage Yield and Quality Components in Alfalfa (*Medicago sativa* L.)

¹B.R. Bakheit, ²M.A. Ali and ³A.A. Helmy

Corresponding Author: B.R. Bakheit, Department of Agronomy, Faculty of Agriculture, Assiut University, Assiut, Egypt

ABSTRACT

The objective of this investigation was to study the effect of two different methods of selection (modified mass and family selection) for crown diameter on forage yield and quality in Ismailia 91 alfalfa variety (Medicago sativa L.). In 2007/2008, two hundred plants (5% intensity of selection) were selected for crown diameter, using the modified mass selection method. Equal seeds from each selected plant were bulked to form each of two selection cycles. Plants selected for C₁ were also raised as half-sib families in 2008/2009 and selection was practiced between and within half-sib families for the best 10 families (5% intensity of selection). Seeds of selected half-sib families and both modified mass selection cycles C₁ and C₂ along with the base population were evaluated for forage yield and quality in 2009/2010 season. The realized gains after the two mass selection were 9.77, 12.68, 14.94, 14.00, 11.34, 14.91 and 12.47% for crown diameter, root length, fresh forage yield, dry forage yield, crude protein, crude fiber and ash (%), respectively over the base population. Gains from family selection as% of base population were 21.24, 16.91, 17.24, 16.00, 16.49,-16.41 and 18.90% for these traits in the same order. All studied traits were positively correlated but the correlation between crude fiber (%) and other traits were negative. However, two half sib-families (No. 1 and 6) were significantly higher than the original population for all studied traits except crude fiber (%). Results suggest that both mass and family selection for crown diameter resulted in great improvement of forage yield and quality. In addition, family selection appeared to be more rewarding than mass selection in improving yield and quality of alfalfa.

Key words: *Medicago sativa* L., mass selection method, family selection method, crown diameter, forage yield and quality components

INTRODUCTION

Alfalfa (Medicago sativa L.) is a major forage crop in most countries around the world. It is cultivated over a wide range of climatic and edaphic conditions ranging from the semi-arid regions to the humid areas. Growth alfalfa increases soil fertility, improves soil structure and reduces erosion (Samac et al., 2003). Developments of adapted high yielding arieties for tolerant biotic and abiotic stress conditions are greatly needed. Breeding methods appropriate for alfalfa improvement have been outlined by several workers. However, himited investigations were reported on selection via root characters specially crown diameter. Most forage legumes persist from one growing season to the next due to their perennial root and crowns (Awad, 2001; Bakheit et al., 2007).

¹Department of Agronomy, Faculty of Agriculture, Assiut University, Assiut, Egypt

²Department of Agronomy, Faculty of Agriculture, South Valley University, Qena, Egypt

³National Forge Crops Res. Program, FCRI, ARC, Giza, Egypt

Crown diameter and root length are genetic traits which control branching, cut weight and number of cutting of alfalfa. The alfalfa crown is important because of its role in the development of new stems after each harvest and for spring regrowth after winter dormancy. Crown morphology is also associated with yield and persistence, since morphology influences the vulnerability of the crown to mechanical injury, winter injury and the attack of several diseases and insects (Marquez-Ortiz et al., 1996). Awad (2001) suggested the efficiency of selection for crown diameter in improving the forage yield and quality in alfalfa. It is clear, therefore, that crown morphology is a key trait for consideration in programs of germplasm improvement and varietal development.

The success of any program of selection to improve crown diameter trait, for indirect selection to forage yield and its components are depend on the genetic variation existing within the initial population or ecotype (Basafa and Taherian, 2009). The heritability of the selected traits, the nature of correlations between different characters and intensity of selection applied are also important for the success of selection. Awad (2001) demonstrated the presence of additive genetic variation and heritance calculations some traits such as root length and crown diameter. The objective of this investigation was to study the effect of two different methods of selection (modified mass and family selection) for crown diameter on forage yield and quality components in Ismailia 91 alfalfa variety during 2007-2010 at Qena, Egypt.

MATERIALS AND METHODS

Parental materials: The starting material used in this study was the seed of alfalfa variety Ismailia 91 obtained from the Forage Crops Section, Field Crops Research Institute, Agriculture Research Center, Egypt. The selection processes and characterization experiments were conducted at the Experimental Farm of South Valley University, Qena, Egypt.

In 2007/2008 growing season, seeds of the original population were sown in 200 rows (200 plots) 2.10 m long. Obtained seedlings were thinned to one plant hill⁻¹. The spacing used throughout this investigation was 10 cm between plants within rows, 20 cm apart except for the last season (evaluation season). All cultural practices were maintained at optimum level for maximum productivity. At the third cut after 145 days from sowing, all plants were dug out including roots and aerial parts (stems and leaves). Crown diameter was measured individually (mm) for each plant. The best plants in each plot or row (one plant/row×200 rows = 200 selected plants = 5% selection intensity) were selected on the basis of the largest crown diameter. Then, they were transplanted in an isolated plot in the field to avoid cross pollination with unselected plants. Seeds were harvested by hand sickle at seed maturity stage mid of June and threshed separately by hand.

Modified mass selection procedure: In 2008/2009, equal parts of seeds from each of the 200 selected plants were bulked together to form the first cycle of modified mass selection (C₁). The same cultural practices, selection procedure and intensity of selection were adopted as described previously. Equal parts of seeds from each of the 200 selected plants were bulked together to form the subsequent generation (C₂). In 2009/2010, for evaluating the response to selection, the base population and two modified mass selection cycles (C₁ and C₂) were compared in a randomized complete block design with three replications. Plot size was 4 m². Seeds were hand-drilled in rows, 20 cm apart, at a seeding rate of 3200 seeds for each replicate. All cultural practices were applied according to the recommendation of alfalfa production. Three cuts were taken from each population. The first, second and third cuts were taken after 70, 110 and 145 days from planting, respectively

and data were recorded for fresh forage yield/cut and were converted into kg m⁻². Also, dry matter was determined from samples of about 300 g plot⁻¹ of fresh forage yield for each cut and were calculated and converted into dry forage yield/cut (kg m⁻²). Crude protein and fiber and ash percentages were determined according to AOAC (1980). At the third cut, plants within an area of 1.0 m² from the middle plot of each replicate were dug to measure the crown diameter and root length.

Family selection procedure: In 2008/2009, the seeds of selected plants for C_1 on the basis of crown diameter were established as half-sib families. Each family consisted of 22 plants. All cultural practices were maintained at optimum levels for maximum alfalfa productivity. The best ten plants in crown diameter trait from the best ten families were selected out of the 200 families (first cycle of the family selection of C_1). They were transplanted in an isolated plot in the field to avoid cross pollination with unselected plants. Seeds were harvested from each selected plant separately. In 2009/2010, the base population and the ten selected families were evaluated in a randomized complete block design with three replications. Plot size was $0.5 \text{ m}^2 (1 \times 1/2 \text{ m})$. Seeds were sown by hand in rows, 20 cm apart at a seeding rate of 400 seeds for each replicate for each family. All cultural practices were applied as recommended. Data for all studied traits were recorded as described for modified mass selection.

Statistical analysis: The analysis of variance and the expected mean squares for all studied traits were performed according to Gomez and Gomez (1984). Mean comparisons were calculated by using revised L.S.D method according to El-Rawi and Khalafalla (1980). The phenotypic (σ^2 p) and genotypic (σ^2 g) variances were calculated according to Al-Jibouri *et al.* (1958). Phenotypic (P.C.V) and genotypic (G.C.V) coefficients of variability were calculated according to Burton (1952). Broad sense heritability (h^2) for each trait was determined as $h^2 = \sigma^2 g/\sigma^2 p \times 100$ (Falconer and Mackay, 1996) and phenotypic (r_p) and genotypic (r_g) correlation coefficients were computed from the components of variance and covariance as outlined by Johnson *et al.* (1955).

The predicted response from selection of the superior 5% plants in C_1 families was estimated as i op h^2 and the correlated response in trait (y) when selection is applied to crown diameter (x) is $C_{ry} = i h_x h_y r_g \sigma_{py}$ according to Falconer (1960) where, $C_{ry} =$ the correlated response of the trait (y), i = the intensity of selection = 2.063, $h_x =$ the square root of the heritability of the trait (x), $h_y =$ the square root of the heritability of trait (y), $r_g =$ the genetic correlation between (x) and (y) traits and $o_{py} =$ the phenotypic standard deviation of trait (y). The realized and correlated gains from selection measured as the deviation percentage of the overall cycle mean from the base population (Falconer, 1989).

RESULTS AND DISCUSSION

Modified mass selection for crown diameter: The differences between means of the base population and two cycles of modified mass selected populations were significant at p<0.01 for the studied traits except crude protein, crude fiber and ash percentages (p<0.05). This indicates that this variation is sufficient in crown diameter a quantitative trait when designing a breeding strategy for cultivar development (Table 1). However, the differences were significant at p<0.01 between the first (C_1) and second (C_2) cycles of modified mass selection for crown diameter and dry forage yield but for root length and fresh forage yield (p<0.05) and insignificant crude protein (%),

Table 1: Mean squares from analysis of variance of crown diameter, root length, fresh and dry forage yield, crude protein, crude fiber and ash of the base population, first (C₁) and second (C₂) cycles of mass selection of alfalfa variety grown in the 2009/2010 season

		Mean squares	Mean squares								
		Root characters	Root characters		Forage yields/cut (kg m ⁻²)		Quality traits (%)				
		Crown diameter	Root	Fresh forage	Dry forage	Crude	Crude				
Source of variance	df	(mm)	length (cm)	yield	yield	protein	fiber	Ash			
Reps	2	0.01	0.20	0.004	0.0001	0.14	0.54	0.59			
Among cycles	2	0.32**	8.35**	0.11**	0.0040**	3.74*	15.86*	1.55*			
Base vs. selection	1	0.39**	13.28**	0.140**	0.0055**	7.36*	25.28*	2.07*			
C1 vs. C2	1	0.24**	3.41*	0.079*	0.0024**	0.12	6.43	0.03			
Error	4	0.01	0.40	0.004	0.0001	0.37	1.78	0.11			

^{*, **}Significant at 0.05 and 0.01 probability levels, respectively

Table 2: Means root length, forage yields and quality for two cycles of mass selection for crown diameter of alfalfa variety grown in the 2009/2010 season

	Root characters		Forage yields/	cut (kg m ⁻²)	Quality traits (%)		
	Crown diameter	Root	Fresh forage	Dry forage	Crude	Crude	
Generations	(mm)	length (cm)	yield	yield	protein	fiber	Ash
Base population	6.65	26.26	2.61	0.50	18.16	30.79	8.74
First cycle of mass selection (C_1)	6.89*	28.08**	2.77*	0.53*	19.93**	28.27*	6.69*
Second cycle of mass selection (C_2)	7.30**	29.59**	3.00**	0.57**	20.22**	26.20**	9.83**
$\mathrm{LSD}_{0.05}$	0.18	1.19	0.11	0.02	1.17	2.54	0.63
$LSD_{0.01}$	0.27	1.81	0.17	0.03	1.79	3.90	0.97

^{*, **}Significant at 0.05 and 0.01 probability levels, respectively

crude fiber (%) and ash (%) (Table 1). These results agree with previous findings of Wang *et al.* (1991), Bakheit and El-Nahrawy (1997), Kimbeng and Bingham (1998), Awad (2001) and Bakheit *et al.* (2007).

Table 2 shows mean values of root characters (crown diameter and root length), forage yields (fresh forage and dry forage yield) and forage quality (crude protein, crude fiber and ash percentages) of the base population and selected generations grown together under common field conditions. The primary criterion of selection was crown diameter and for this trait changes in the first (C₁) and second (C₂) selected progenies were consistently in the direction of selection. Means of both C₁ and C₂ cycles were significantly greater than the C₀ for all studied traits, while it were significantly lower than the C₀ for crude fiber (%). However, Means of the C₂ cycle were significantly higher than the C₁ for root characters and forage yields but it were insignificantly for the quality components (Table 2), reflecting the response to selection in improving crown diameter. The realized and correlated gains from selection estimated as the percentage deviation of the mean of the base population are shown in Table 3. The results indicate that direct selection was effective in improving crown diameter by 9.77% after the second cycle of modified mass selection. This gain was accompanied by 12.68, 14.94, 14.00, 11.34 and 12.47% gain in root length, fresh forage yield, dry forage yield, crude protein and ash (%), respectively. However, the increase in crown diameter caused decrease in crude fiber percentage (-14.91%). Saindon et al. (1991), Marquez-Ortiz et al. (1999) and Awad (2001) found that the best indirect selection to improve forage yield was crown

Table 3: Realized gains from modified mass selection for crown diameter in the two cycles of selection measured in percentage from the base population

			Root characters		Forage yields/o	cut (kg m ⁻²)	Quality traits (%)		
			Crown diameter	Root length	Fresh forage	Dry forage	Crude	Crude	
Selection criteria	Item		(mm)	(cm)	yield	yield	protein	fiber	Ash
Crown diameter (mm)	C_0	C_1	3.61*	6.93**	6.13 *	6.00*	9.75**	-8.18*	10.87*
		C_2	9.77**	12.68**	14.94**	14.00**	11.34**	-14.91**	12.47**

^{*,**}Significant at 0.05 and 0.01 probability levels, respectively

Table 4: The analysis of variance of root characters, forage yields and quality for the base population and ten selected families of alfalfa variety grown in the 2009/2010 season

		Mean squares	Mean squares								
		Root characters		Forage yields/cut (kg m ⁻²)		Quality traits (%)					
		Crown diameter	Root length	Fresh forage	Dry forage	Crude	Crude				
Source of variance	df	(mm)	(cm)	yield	yield	protein	fiber	Ash			
Reps	2	0.11	7.59	0.02	0.004	10.16**	4.40	0.54			
Families	10	3.20**	54.01**	0.38**	0.013**	14.47**	24.91**	3.24**			
Base vs. selected fam.	1	5.45*	53.76*	0.54**	0.020*	24.62**	69.64**	7.45**			
Between selected fam.	9	2.91*	54.03**	0.36**	0.013**	13.35**	19.99**	2.78**			
Error (families)	20	0.86	8.00	0.03	0.003	1.43	2.32	0.48			
Error (sel. families)	18	0.91	8.66	0.03	0.003	1.58	2.22	0.52			

^{*,**}Significant at 0.05 and 0.01 probability levels, respectively. df = Degrees of freedom

diameter. However, Chloupek (1984) and Chloupek et al. (1999) also reported that the best indirect selection criterion for yield was the root system size.

These results reflect the efficiency of modified mass selection for crown diameter on improving root length, forage yield and forage quality of alfalfa.

Family selection: There were significant (p<0.01) differences among families for all studied traits (Table 4). Differences between the ten selected families and the base population were significant at p<0.01 for the studied traits except crown diameter, root length and dry forage yield (p<0.05). However, differences among the selected families for these traits were significant at p<0.01 except for crown diameter (p<0.05).

Data presented in Table 5 show means of root characters, forage yields and quality traits for the selected families and their base population. The range in crown diameter of the ten selected families was 6.67 to 9.67 mm with an average of 8.07 mm. This variation is sufficient to consider crown diameter a quantitative trait when designing a breeding strategy for cultivar development. Four families were significantly larger for crown diameter than the base population after one cycle of family selection. The root length ranged from 23.33 to 38.33 cm with an average of 30.70 cm and four families (No. 1, 3, 4 and 6) were significantly longer than the base population. With respect to fresh forage yield and dry forage yield, it ranged from 2.49 to 3.54 kg m⁻² with an average of 3.06 kg m⁻² and from 0.48 to 0.67 kg m⁻² with an average of 0.58 kg m⁻², respectively. Furthermore, all families except three families (No. 2, 8 and 9) for fresh forage yield and half families (No. 1, 4, 6, 7 and 10) for dry forage yield were significantly higher than the base

Table 5: Means of root characters forage yields and quality for the base population and the ten selected families of alfalfa variety grown in the 2009/2010 season

	Root characters		Forage yields/o	eut (kg m ⁻²)	Quality traits (%)			
Family	Crown diameter (mm)	Root length (cm)	Fresh forage yield	Dry forage yield	Crude protein	Crude fiber	Ash	
1	9.00*	35.33**	3.34**	0.64**	23.78**	23.19**	11.69**	
2	6.67	30.67	2.89	0.55	20.01	25.70**	10.12	
3	7.33	33.33**	2.98*	0.57	21.94**	26.09**	9.94	
4	7.67	31.33*	3.09**	0.59*	20.28*	27.77*	10.25	
5	8.33	29.67	2.91*	0.55	21.59**	27.00**	10.75**	
6	9.67**	38.33**	3.54**	0.67**	23.98**	22.56**	11.14**	
7	8.67*	28.67	3.35**	0.63**	22.14**	24.22**	10.44	
8	8.00	29.33	2.49	0.48	18.77	28.66	8.65	
9	6.67	27.00	2.61	0.50	17.32	29.74	9.35	
10	8.67*	23.33	3.37**	0.64**	22.14**	22.47**	11.63**	
Base population	6.65	26.26	2.61	0.50	18.19	30.79	8.74	
Selec. fam. mean	8.07	30.70	3.06	0.58	21.19	25.74	10.40	
$\mathrm{LSD}_{0.05}$	1.83	4.97	0.29	0.09	2.01	2.54	1.23	
$LSD_{0.01}$	2.71	7.05	0.40	0.13	2.80	3.55	1.74	

^{*, **}Significant at 0.05 and 0.01 probability levels, respectively

population after one cycle of family selection. In addition, crude protein and ash percentages for the ten selected families ranged from 17.32 to 23.98% with an average of 21.19% and 8.65 to 11.69% with an average of 10.40%, respectively. Furthermore, eight families for crude protein (%) and four families for ash (%) were significantly higher than the base population after one cycle of family selection. The range in crude fiber (%) among selected families was 22.47 to 29.74% with an average of 25.74%. Crude fiber (%) of most families was significantly lower than the base population after one cycle of family selection.

These results demonstrate that the families which outyielded the base population for forage yield were the same which were superior for crown diameter. These results could be due to the high correlation between forage yields and crown diameter. Two families i.e., 1 and 6 were 35.34 and 45.41% larger than the base population in crown diameter, 34.54 and 45.96% longer in root length, 27.97 and 35.63% higher in fresh forage yield, 28.00 and 34.00% in dry forage yield, 30.73 and 31.83% in crude protein (%)-, 33.75 and 27.46% in ash (%) and-24.68 and-26.73% lower in crude fiber (%), respectively.

These findings suggest that indirect selection for forage yield and quality components was effective in improving this population. Some investigators found similar results such as Bakheit (1989), Awad (2001) and Bakheit *et al.* (2007).

The phenotypic and genotypic variances expressed as PCV% and GCV% (Table 6) were 12.33 and 10.14% for crown diameter, 13.82 and 12.67% for root length, 11.44 and 10.74% for fresh forage yield, 10.84 and 9.46% for dry forage yield, 9.91 and 9.35% for crude protein (%), 10.03 and 9.46% for crude fiber (%) and 9.22 and 8.33% for ash (%), respectively. These results reveal sufficient genotypic variability among selected families after one cycle of family selection for crown diameter.

Broad sense heritability (Table 6) was high in magnitude for root length (83.91%), fresh forage yield (92.14%), crude protein (%) (88.09%), crude fiber (%) (88.89%) and ash (%) (81.52%). However, heritability for crown diameter and dry forage yield were smaller than quality traits

Table 6: Phenotypic $(\sigma^2 p)$ and genotypic $(\sigma^2 g)$ variances, phenotypic (PCV%) and genotypic (GCV%) coefficients of variability, heritability% (H) and realized responses

	Root characters		Forage yields/cu	t (kg m ⁻²)	Quality traits (%)		
	Crown diameter	Root length	Fresh forage	Dry forage	Crude	Crude	
Estimate	(mm)	(cm)	yield	yield	protein	fiber	Ash
$\sigma^2 \mathbf{p}$	0.99	18.01	0.12	0.004	4.45	6.66	0.92
$\sigma^2 \mathbf{g}$	0.67	15.12	0.11	0.003	3.92	5.92	0.75
PCV (%)	12.33	13.82	11.44	10.84	9.91	10.03	9.22
GCV (%)	10.14	12.67	10.74	9.46	9.35	9.46	8.33
H (%)†	67.68	83.91	92.14	75.00	88.09	88.89	81.52
Predicted gains							
Unit	1.40	3.12	0.54	0.09	3.32	-4.06	1.42
% of base population	20.89	10.17	20.82	17.20	18.25	-13.19	16.22
Realized gains							
Unit	1.41*	4.44 *	0.45**	0.08*	3.00**	-5.05 **	1.65**
% of base population	21.24*	16.91*	17.24**	16.00*	16.49**	-16.41**	18.90**
Revised $LSD_{0.05}$	1.35	3.68	0.21	0.07	1.48	1.89	0.90
Revised $LSD_{0.01}$	1.99	5.22	0.29	0.10	2.05	2.63	1.28

[†] H, Broad sense heritability

suggesting more environmental influences on crown diameter and dry forage yield. Similar results were reported by Bakheit (1989), Marquez-Ortiz *et al.* (1999), Awad (2001) and Bakheit *et al.* (2007).

Predicted and realized gains from direct selection for crown diameter and correlated responses in root length, forage yields and quality components are presented in Table 6. The results indicated that after one cycle of family selection, the realized gain for crown diameter reached 21.24% from the base population. Likewise, the expected gain from selection based on the superior 5% plants in C₁ family selection was 20.89%. Furthermore, favorable increase in root length (16.91%), fresh forage yield (17.24%), dry forage yield (16.00%), crude protein (16.49%) and ash (18.90%) and favorable decrease in crude fiber (%) (-16.41%) were obtained (Table 6). Comparison of the expected and realized gain from selection shows a quite good agreement between expected and realized gain in crown diameter and dry forage yield. However, the realized gain for root length, crude fiber (%) and ash (%) was higher than the expected one but for fresh forage yield and crude protein (%), the expected response was higher than the realized one. These results are in line with those reported by Barnes et al. (1983), Bakheit (1989), Bakheit and El-Nahrawy (1997), Awad (2001) and Bakheit et al. (2007).

The results of phenotypic (r_p) and genotypic (r_g) correlations between each pair of the seven traits (Table 7) showed that the genotypic correlation coefficients were higher than their corresponding phenotypic correlations. Also, high genotypic correlations suggested that there was inherent relationship between traits. Thus, all seven traits had positive correlation with each other, except crude fiber (%), however, crown diameter and each of fresh forage yield, dry forage yield and quality traits (crude protein and ash%) were very closely related (0.99), indicating that selection for changes in crown diameter would modify expression of the other traits. Root length was highly positively correlated with dry forage yield but it was moderately correlated with both fresh forage yield and crude protein ratio. However, a weak correlation was found between root length and ash percentage (0.19). These results confirmed those obtained from the analysis of

Table 7: Phenotypic and genotypic correlation coefficients among pairs of traits of the ten selected families of alfalfa variety

	Root characters		Forage yields/c	ut (kg m ⁻²)	Quality traits (%)		
Trait	Crown diameter	Root length	Fresh forage yield	Dry forage yield	Crude protein	Crude fiber	Ash
Crown diameter (mm)	, ,	0.320	0.752	0.788	0.813	-0.758	0.689
Root length (cm)	0.473		0.537	0.641	0.534	-0.272	0.190
Fresh forage yield kg m ⁻²	0.916	0.675		0.960	0.871	-0.856	0.827
Dry forage yield kg m ⁻²	0.974	0.918	0.999		0.933	-0.890	0.868
Crude protein (%)	0.991	0.602	0.978	0.914		-0.890	0.835
Crude Fiber (%)	-0.990	-0.271	-0.980	-0.999	-0.920		-0.854
Ash (%)	0.970	0.194	0.998	0.994	0.911	-0.993	

Phenotypic (above) and genotypic (below diagonal) correlation coefficients

variance of Table 3. Crude fiber percentage was highly negatively correlated with all traits except root length. These results are in line with those obtained by Montpetit and Caulman (1991), Awad (2001) and Bakheit *et al.* (2007).

CONCLUSION

The present study suggested that both mass and family selection for crown diameter resulted in great improvement of forage yield and quality. In addition, family selection appeared to be more rewarding than mass selection in improving yield and quality components of alfalfa.

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