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EVALUATING THE PERFORMANCE OF AUSTRALIAN ANNUAL MEDICS IN SUB-TROPICAL AND SUB-HUMID ECOLOGICAL ZONES OF PAKISTAN

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The study was conducted to test the performance of *Medicago* varieties in sub-tropical and sub-humid ecological zones of Pakistan on the basis of dry matter accumulation, root nodulation and nitrogen fixation. For this purpose, certified uninoculated seeds of *Medicago* varieties were sown in the first week of October 2005 at National Agricultural Research Centre (NARC), Islamabad. The study reveals that *Medicago blancheana*, var. SA-3410 and *Medicago rugosa* Var.SA-5814 were densely covered with active nodules. Both varieties also had the highest N-content in their tissues. *Medicago arabica* var. SA-2355, var. SA 2041, *M. liltoralis* var 'SA-2144' *M. murex* var. SA-4000, *M. polymorpha* var. SA-2167, var. SA-3836, *M. rotata* var-2145, *M. Scutellata* var. SA-2082 and var. SA-7620 yielded more than 5 t. ha^{-1} dry matter. Hence, these varieties were regarded as promising for rangelands and marginal croplands located in sub-humid and sub-tropical ecological zones of Pakistan.
Keywords: Annual Australian medics, matter yield, root nodulation, nitrogen fixation

INTRODUCTION

Nitrogen is a major limiting nutrient in grasslands, but use of nitrogenous fertilizer on grasslands has been low in Australia due to high costs in relation to the value of livestock products and since there are problems of acidification and nitrate contamination in water supplies. The use of nitrogen fertilizer remains restricted to situations offering high financial returns, such as for winter feed supplies in dairy farms in the sub-tropics. (Alferdo *et al.* 2004).

The experiments in southern Australia provided a model for northern Australia and optimistic estimates were made of the area suitable for sown pastures in the tropics including an area of 50-60 m. ha. in Queensland (Davies and Elyes, 1965; Ebershon and Lee, 1972; Weston *et. al.,* 1981), although later estimates were 22 m. ha. (Walker and Weston, 1990). Initial hopes for twining legumes (e.g *Medicago rugosa* and *Medicago arabica* sp.) were not fulfilled, although some use of these species continues. These elevated growing points are sensitive to grazing (Clements, 1996) and these species lack persistence under heavy grazing. There are successful developments based on different Australian annual medics.

In Mediterranean region, an intensive research has been conducted on bur medic (*Medicago polymorpha*) in order to collect and characterize accessions (del Pozo *et al*. 2002a, 2002b; Paredes *et al*. 2002) and to select cultivars (Ovalle *et al.* 2001; del Pozo *et al.* 2001) and rhizobium. Cultivars of various legume species have been developed in Australia during the recent years (Dear *et al.* 2002; Loi *et al*. 2005. Since 1991, 49 new pasture legume cultivars have been developed by public institutions (Nichols *et al.* 2006).

There have been positive changes for the use of legumes such as increased dry matter production, soil improvement, increased root nodulation and nitrogen fixation. However, legumes can also have negative impacts such as soil acidification. So there will be a continuing need for research and development to overcome problems that arise as well as for proper management. Thus cultivating legumes is not simply a matter of selecting and sowing species and expecting them to survive and produce with little management.

The present study was conducted to assess the performance of Australian originated *Medicago* varieties on the basis of dry matter production, root nodulation and nitrogen fixation in the sub-tropical and sub-humid ecological zones in Pakistan.

MATERIALS AND METHODS

The study was conducted at National Agricultural Research Centre (NARC), Islamabad, which is located in the sub-tropical and sub-humid ecological zones. The average annual rainfall recorded at NARC was about 1132 mm with maximum (270mm) during August and minimum (04 mm) during November. Minimum temperature seldom falls below zero during January, while the maximum temperature up to 39^0C was recorded in June (WRRI, 2005). The soils were non-saline loamy with 0.53% organic matter, 8.4 pH and 0.53 dsm^{-1} electrical conductivity.

Certified uninoculated seeds of 37 *Medicago* varieties received from South Australia were sown in 4 x 4 m plots in the first week of October, 2005 after a heavy rainfall. The sowing was conducted with the aid of a hand-pulled drill. Row spacing was 30 cm. No irrigation and fertilizer were applied. Randomized Complete Block Design (RCBD) with four replications was applied. The first harvesting of plants was at 50 % flowering stage and second at pod filling stage. At each harvest, five plants from each plot were uprooted. The roots were washed under running tap water to remove adhering soil particles. Nodules were counted and were excised from the roots. The dry weight of nodules was recorded after oven drying at 60 ^{0}C for 48h. The plants were ground through a 40 mesh screen. Nitrogen content was determined using the Micro-Kjeldhal method (AOAC, 1994). The data was subjected to analysis of variance (ANOVA) and means were separated using least significant differences (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

The nitrogen accumulates in small appendages called “nodules” which are formed on the roots of legume plants (Dorland *et. el*., 2001). During the study, the highest number of root nodules (89 plant^{-1}) at 50 % flowering stage were recorded in *M. rugosa* var. “SA-5814”. Eleven varieties were well nodulated with more than 15 root nodules per plant. Twenty varieties had 5-10, whereas six varieties had less than five nodules per pant (Table 1).

At pod filling stage, *M. blancheana* var. SA-3410, produced 128 root nodules per plant. Nine varieties including *M. blancheana* var. SA-3410 had more than 20 root nodules per plant. Seventeen varieties were moderately nodulated with 10-20 root nodules per plant while eleven varieties had less than 10 root nodules per plant (Table 1)

At 50% flowering stage, *M. arabica* var, SA-2334, had the highest dry weight of nodules (18.40 mg plant^{-1}) while *M. polymorpha* var. SA-4408, had only 0.20 mg plant^{-1}. Ten varieties had more than 4 mg plant^{-1} dry weight of nodules, in 12 varieties the value ranged from 2 to 4 mg and 15 varieties had dry weight of nodule less than 2 mg plant^{-1}. At pod filing stage, *M. rugosa* var.1805, had the highest (28.92mg) and *M. tornata* var. SA-2698, had the lowest (0.64 mg) root nodule dry weight per plant. Twenty varieties had root nodules weighing more than 6mg, in twelve varieties range was 3-6 mg and five varieties had less than 3 mg nodule dry weight per plant.

Contrasting variations regarding the effectiveness and infectiveness of soil Rhizobia in forming nodules in various *Medicago* species were noted in this study. Nodule formation and nitrogen fixation involve genotype interaction between the legume and invading Rhizobium in which the plant plays a dominant role. These results are in consistent with the findings of Nandasena *et al.* (2006, 2007).

The colour of root nodules varied from white to pink at 50 % flowering stage whereas at pod filling stage, some nodules were dark brown in color and were degenerating. At both the harvesting stages, most varieties had elongated and delicate branched nodules. The repeated branch of nodules resulted in the formation of relatively large coralloid structures. The morphological features of nodules resembled the nodules described (Bose and Balakarishnan, 2001). The shapes of the nodules described by the researchers were used as a guide for recognizing nodular structure in the present study. The nodules first appeared as a small lateral swelling on the roots, but later on new lobes were produced on their apices resulting in the formation of clustered or coralloid forms.

The nodules were observed to be pink because of hemoglobin and were regarded as effective whereas white nodules were considered as ineffective. The nodules at first harvest were white to pink but at second harvest these were brown/pink in colour. This may be due to the fact that at early stages nodules were in the developing stage and did not fix nitrogen whereas, at second harvest fully matured nodules were functional as assessed by their colour. However, in some instances nodules at second harvest were also found to be degenerating.

At 50 % flowering stage N-content plant^{-1} was highest in *M. rugosa* var.SA-5814 (292.9 mg) and lowest in *M. polymorpha* var. SA-1327 (32.5 mg). Five varieties had more than 200 mg, twelve had 100 to 200 mg whereas twenty varieties had less than 100 mg N-contents plant^{-1} (Table 1). At pod filling stage, highest N-content per plant was found in *M. blancheana* var. SA-3410 (481.1 mg) and lowest in *M.rugosa* var.5814 (217 mg) N-contents of eight varieties were more than 200 mg, ten varieties were in the range of 100-200 mg and in nineteen varieties were less than 100 mg plant^{-1}.

At 50% flowering stage, highest N-content per nodule was found in *M. arabica* var.SA-2334 (0.74 mg) and lowest in *M. orbicularis* var. SA-2552 and *M.polymorpha* var. SA-2167 (0.02 mg). Nine varieties had more than 0.30 mg N-content nodule^{-1}, in other twelve varieties; its range was from 0.15 to 0.30 while sixteen varieties had N-content less than 0.15 mg nodule^{-1}. Ru and Fortune (2000) outlined that varieties in both DMD and nitrogen concentration among plants parts offers a potential for selection of leafy cultivars for high nutritional characteristics.

At pod filling stage, root nodules of *M. blancheana* var.SA-3410, had the highest N-content (2.29 mg) and *M. orbiclaris* var.SA-2552 and *M. tornata* var.SA-2698 had the lowest N-contents. In eleven varieties, it was between 0.20 and 0.40 mg and remaining seventeen varieties had less than 0.20 mg N-contents per root nodule.

Medicago disciformis var.SA-2355 produced the highest (8.50 t. ha^{-1}) dry matter while *M. rugosa* var.SA-5814 produced only 0.1 t/ha. Ten promising varieties produced the dry matter more than 5 t.ha^{-1}. Fifteen varieties produced the

Table1. Nodulation, N-contents and dry matter yield in *Medicago* varieties at 50 % flowering and pod filling stages at NARC during 2004-05

S. No.	Species/Variety	Harvest I (At 50%Flowering)				Harvest II (At pod filling)				Dry matter yield t.ha^{-1}
		No. of nodules	Nodule Dry wt. (mg)	N-content(mg) Plant	N-content(mg) Nodules	No of nodules	Nodule Dry wt (mg)	N-content(mg) Plant	N-content(mg) Nodule	
1	*M.aculeata*- SA-3059	4.8 c	0.96 i	86.7 de	0.06 f	66.8 ab	6.06 bc	46.6 e	0.91 ab	3.50 c
2	*M.aculeata*- SA-4151	2.8 j	0.70 i	197.0 bc	0.08 f	1.6 h	3.32 de	184.9 b	0.25 bc	2.20 d
3	*M.arabica*- SA-2041	6.8 gh	2.44 de	178.4Bc	0.26 d	4.8 f	4.44 cd	47.67 e	0.08 de	6.25 a
4	*M.arabica*- SA-2334	10.0 e	18.40 a	47.5 f	0.74 a	17.6 bc	6.03 bc	28.40 g	0.18 cd	4.85 b
5	M.*blancheana*- SA-3410	59.8 b	11.26 b	160.3 c	0.57 b	128.6 a	4.92 c	481.1 a	2.29 a	3.50 c
6	*M.disciformi*- SA-2355	15.2 d	4.66 c	82.8 e	0.65 a	31.2 b	6.38 bc	41.8 f	0.23 c	8.20 a
7	*M.intertexta*- Sa-2071	8.4 fg	1.34 fg	162.8 c	0.06 f	4.2 fg	1.06 f	109.9 c	0.03 e	4.50 b
8	*M.intertexta*- SA-2377	9.8 ef	3.32 d	236.9 b	0.35 c	11.2 e	4.30 cd	131.0 bc	0.29 bc	4.50 b
9	*M.littoralis*- SA-2144	2.6 j	0.82 c	48.3 f	0.09 f	14.0 c	3.08 e	45.2 f	0.08 de	5.10 a
10	*M.littoralis*- SA-3072	18.8 cd	3.92 d	87.7 de	0.13 e	19.0 bc	3.90 d	260.2 ab	0.20 c	2.25 d
11	*M.littoralis*- SA-3838	20.2 c	3.92 d	141.4 cd	0.27 d	25.2 b	6.90 b	272.4 ab	0.31 bc	4.00 bc
12	*M.littoralis*- SA-4015	20.8 c	6.32 bc	84.0 de	0.36 c	12.4 cd	3.40 dc	140.6 b	0.16 cd	1.50 e
13	*M.murex*- SA-2197	8.6 f	2.34 e	84.9 de	0.13 f	13.4 cd	8.70 b	261.5 ab	0.42 b	3.15 c
14	*M.murex*- SA-3999	12.0 de	0.96 i	54.4 f	0.06 f	11.4 cd	4.86 cd	51.7 e	0.10 d	2.50 d
15	*M.murex*- SA-4000’	14.6 d	2.14 ef	105.5 d	0.11 f	19.2 b	5.90 bc	140.2 b	0.20 c	6.75 a
16	*M.orbicularis*- SA-1804	24.6 bc	4.22 cd	87.9 de	0.26 d	17.6 bc	3.04 e	92.8 cd	0.13 d	4.60 b
17	*M.orbicularis*- SA-2550	6.2 gh	1.28 fg	107.0 d	0.13 e	7.8 f	6.96 b	21.2 g	0.41 b	3.80 bc
18	*M.orbicularis*- SA-2552	1.8 j	0.21 j	200.0 bc	0.02 g	4.2 fg	0.68 f	86.9 d	0.02 e	3.15 c
19	*M polymorpha*- SA-1327	8.2 fg	1.54 f	32.5 h	0.11 f	14.8 c	3.04 e	34.5 f	0.10 d	3.50 c
20	*M polymorpha*- SA-2167	3.6 I	0.34 i	70.7 ef	0.02 g	13.2 cd	3.46 de	49.4 e	0.10 d	5.20 a
21	*M polymorpha*-SA-3429	4.0 I	0.52 i	49.3 f	0.03 g	6.0 f	1.69 f	48.5 e	0.05 e	2.25 d
22	*M.polymorpha*-SA-3836	16.2 d	2.88 de	212.2 bc	0.17 e	42.6 ab	10.78 ab	168.1 b	0.52 b	5.20 a
23	*M24.polymorpha*- SA-4043	9.2 f	1.34 fg	41.2 fg	0.15 e	8.8 ef	4.28 cd	54.9 e	0.20 c	4.00 bc
24	*M.polymorpha*- SA-4408	7.6 fg	0.20 j	40.7 fg	0.43 c	23.4 b	7.74 b	70.2 d	0.35 bc	2.00 de
25	*M.rotata*- SA-2145	23.8 c	4.22 cd	141.9 cd	0.27 cd	15.6 c	3.82 d	34.6 f	0.10 d	5.25 a
26	*M. rugosa* -SA-1805	24.4 bc	13.80 b	79.5 c	0.63 b	27.4 b	28.92 a	105.7 c	1.42 ab	3.00 cd
27	*M .rugosa*- SA-5814	89.0 a	5.64 bc	292.9 a	0.34 c	117.6 a	5.76 c	21.7 g	0.17 cd	0.10 j
28	*M.scutellata*- SA-2082	5.6 I	1.36 fg	187.1 bc	0.17 bc	8.8 ef	10.68 ab	134.3 bc	0.46 b	5.50 a
29	M.scutellata- SA-4331	8.6 f	2.02 ef	47.3 f	0.12 f	17.0 bc	6.06 bc	46.6 e	0.18 cd	2.85 d
30	*M.scutellata*- SA-6514	9.2 f	1.66 f	168.8 c	0.08 f	7.4 f	2.50 ef	48.9 e	0.08 de	5.00 ab
31	*M.scultellata*- SA-7620	12.0 e	4.64 c	234.4 b	0.22 e	13.8 cd	3.76 d	119.9 c	0.22 c	8.36 a
32	*M .scutellata*- SA-8042	11.8 e	2.36 e	115.9 d	0.17 e	11.2 e	4.94 c	216.3 b	0.23 c	3.00 cd
33	*M.tomata*- SA-2698	11.0 e	3.92 d	82.3 e	0.24 d	0.8 j	0.64 f	22.4 g	0.02 e	2.95 cd
34	*M.tomata*- SA-2699	6.1 gh	1.28 fg	80.3 e	0.13 f	9.2 e	5.18 c	257.0 ab	0.50 b	4.50 b
35	*M.tomata*- SA-4970	9.2 f	2.32 e	171.7 c	0.15 e	17.6 bc	3.76 d	165.1 b	0.12 d	1.10 e
36	*M.truncatula*- SA-4783	10.2 e	5.12 c	77.9 ef	0.37 bc	10.0 e	5.68 c	245.0 ab	0.24 c	2.20 d
37	*M.turbinata*	19.4 cd	3.18 d	62.4 ef	0.19 e	34.4 b	8.76 b	95.7 cd	0.40 b	2.00 de

Values followed by the same letter (s) are statistically similar at P=0.05 level of significance.

dry matter in the range of 3-5 t. ha^{-1} and were regarded intermediate, whereas, twelve varieties were found to be low yielding with dry matter less than 3 t ha^{-1}. N-content was found to be significantly correlated with plant dry weight (r=0.887; p<0.001 for harvest I and r=0.798, p<0.001 for harvest II). Bur medic stubbles showed a better chemical composition as compared to standing hay of fertilized and unfertilized natural pasture based on grasses (Fois *et al.* 2000)

The relationship between dry matter yield and total nitrogen were highly correlating in effectively nodulated plants. However, some degree of differentiation may be lost, when using weight, due to greater concentration of nitrogen in the tissue of the sample plant.

CONCLUSION

It could therefore be concluded that *M. blancheana* var. SA-3410 and *M. rugosa* var.SA-5841 were well nodulated with active nodules. These varieties also had highest N-content in their tissues. Annual medics yield of dry matter up to 5 t. ha^{-1} under rainfed conditions can be regarded as high yielding species. In this study *M.arabica* var. SA-2355, SA-2041, *M. littoralis* var.SA-2144, *M.murex* var.SA-4000, *M.*

polymorpha var.SA-2167, var.SA-3836' *M rotata* var. SA-2145, *M.scutellata* var. SA-2082, var. SA-6514; and var.SA-7620; yielded dry matter more than 5 $t.ha^{-1}$. These varieties could be therefore regarded as promising varieties for rangelands and marginal croplands located in sub-humid and sub-tropical zones of Pakistan.

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