

STUDY OF NITROGEN FIXATION AND NODULATION IN ANNUAL MEDIC(*MEDICAGO RIGIDULA*) IN INOCULATION WITH FOREIGN AND INSIDE ROOT SYMBIOTIC BACTERIA

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ABSTRACT

Annual species of *Medicago* are important pasture legumes in western parts of Iran. Their productions are affected by suitable soil *Rhizobium meliloti* strains and environmental conditions. The principle objective of this study was to find a suitable *Rhizobium meliloti* strain(s) for *Medicago rigidula*.

in the greenhouse conducted in 2006 to determine nodulation, and nitrogen fixation of *M. rigidula* inoculated with five *Rhizobium meliloti* strains (Domestic from Mahabad, M3, M15, M29 and WSM540).

Symbiotic effectiveness was measured by nodule number and nitrogen fixation by subtracting seed N from total plant N in minus-nitrate treatment at the end of the experiment. The *Rhizobium meliloti* strain "Domestic from Mahabad" was found to be more effective than the M3, M15, M29 and WSM540 in terms of nodulation, dry matter production and N₂ fixation. This may be due to cold resistance of this *Rhizobium* strain. While nodule number is a very important consideration of symbiotic effectiveness it should be combined with total dry matter and total nitrogen in the evaluation of strains. *Rhizobium meliloti* strain "Domestic of Mahabad" is recommended for inoculation of *M. rigidula* in western parts of Iran.

Keywords: *M. rigidula, Rhizobium, Nodulation, Biological Nitrogen Fixation*

INTRODUCTION

The growing increase in application of nitrogen chemical fertilizer as to increase cereal yield is perilous. No doubt that nowadays with the advancement of science and technology in the field of plant biology and physiology, the biological N fixation by the legume plant in rotation of wheat is of importance as an inevitable strategy in sustainable and novel agriculture.

It is the current practice in cereal-producing areas of West Asia and North Africa to grow barley or wheat in rotation with fallow. It may be beneficial to replace that fallow with self-regenerating clovers or annual medics (McWilliam 1982). This integrated system, known as ley-farming, is well developed in those regions of southern Australia that have a Mediterranean-type climate (Cocks et al. 1980).

Annual medics have been valued for their capacity to provide N needs through biological nitrogen fixation (BNF). Effectively nodulated medics may fix 60 kg N/ha/yr (Koocheki and Astarayi 1996). In addition to supplying N for the current medic crop, BNF has also been valued for providing N to the succeeding rotating crops. Two characteristics of annual medics – reducing fertilizer needs and improving soil structure- are of particular importance to developing countries where agricultural production is often limited by an economical supply of N fertilizer (Burton 1981). There are about 25 million hectares (Mha) fallow lands in Iran, Iraq, Syria and Turkey, 19 Mha of them are suitable for cultivation of annual medics. According to Carter (1981) there are about seven Mha fallow lands in Iran, those lands are often allotted to cultivation of legume.

Annual medics are found in almost all region of Iran indicating that these plants are appropriate for cultivation in Iran. It was observed during the work that annual medics especially *M. rigidula* required specific strains of *Rhizobium meliloti* in order to fix N. Indeed some soils in Iran, climatically and edaphically suitable for the plant, contain no appropriate rhizobia. Our objectives were to define the degree of symbiotic specificity within the *Medicago-Rhizobium meliloti* association and to identify highly effective strains of rhizobia that might be used for inoculating seeds of medicago.

MATERIALS AND METHODS

In order to study the impact of Rhizobium strains on biological nitrogen fixation (BNF) in annual medic an experiment was conducted at Islamic Azad University branch of Mahabad 2007 .

At first a simple Rhizobium strain was excavated purified at the height of 1500 m above sea level and then it was cultured and purified on the medium (yeast, agar, manitol) with foreign strain M₃, M₂₉, M₅₄₀, M₁₅ (Vincent, 1970). The seed of two annual medic species, *M. rigidula* and *M. polymorpha*, were planted in vases consisting of quartz and then they were irrigated with distilled water. After the seedling appeared the plants were inoculated with 1ml suspension of rhizobium.

The check (no inoculation) along with other treatments were irrigated with Hoglant solution (Heidari, 1994). In each vase as many as 8 plants (based on plant population of 1200 plant /m²) were conserved for 60 days under greenhouse and controlled condition. Then the plants were harvested and the following traits were measured: stem dry weight, stem weight, root dry weight, percentage of nitrogen and protein.

RESULTS

The impact of rhizobium strains of annual medic species and their interaction on nitrogen content in the aerial parts and their dry matter was significant at P<0.01. The ability of annual medic species to make a coexistence with the rhizobium differed.

M.rigidula and *M.polymorpha* had the greatest level of coexistence with local strain regarding dry weight production of stem (26.75 and 21.15 mg respectively). The results of data mean comparison strain that the greatest root dry weight in *M. rigidula* belonged to the coexistence with strain M₅₄₀ (16.66mg) having a significant difference with other strains and the check but, the greatest root dry weight in *M.polymorpha* belonged to the coexistence with local strain (9.66mg) having no difference with strain M₃. The highest amount of protein and N% in *M. rigidula* and *M.polymorpha* belonged to the coexistence with the local strain (14.35%, 2.16 gr/dry matter for *M.polymorpha* respectively).

DISCUSSION

The annual alfalfa, *M.rigidula* is one of grass legume. That has a very little response to *Rhizobium meliloti* and the latter produces nodule and fixes nitrogen. One reason is because of the lack of conformity between them, so that *M.rigidula* is native to cold and temperate zones but most of alfalfa strains are special to tropical zones.

As the legumes are divided into two groups of temperate and tropical legumes, the rhizobium strains can also be grouped. It is recommended that as to increase the number of nodules and N fixation in cold zones legumes and rhizobium be collected. The amount of fixed N by *M.rigidula* and the local strain in greenhouse is approximately 6 mlgr per plant if the number of plants per unit area is 1200, approximately 7 gr/m of net N and 2gr of net N in the control plant without using rhizobium will be fixed which the difference between them is 5gr (50kg/ha).

Species	Strain	Cotrol	M ₂₉	M ₁₅	WsM ₅ 40	M ₃	Loca ly	Traits were measured						
	rigidula	0.025	e	0.873	de	0.413	e	0.336	e	2.233	b	1.4	cd	nodule number
	polymorpha	0.746	de	0.883	de	1.26	cd	1.5	cd	1.9	bc	6.43	a	nodule number
	rigidula	12.66	ef	12.67	e	11.5	ef	16.67	d	11.83	ef	21.1 5	c	dry stem weight
	polymorpha	10.88	f	17.23	d	25.93	a	20.16	c	26.75	a	24	b	dry stem weight
	rigidula	5.9	g	9.33	de	8.5	df	7.83	ef	9.66	d	9.67	d	root dry weight
	polymorpha	7.16	fg	9.5	de	14.73	b	16.67	bc	13.91	bc	12.8 3	c	root dry weight

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