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Nodulation of Medicago Sativa L. by Different Rhizobium Strains in the Sultanate of Oman

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تكوين العقد الجذرية لنبات البرسيم بواسطةٍ أنواع مختلفة من بكتيريا العقد الجذرية بسلطنة عمان

عبد القادر الزين الشفيع ، وريجنالد فيكتور ، ونوره المقبالي

خلاصة : لم تجد دراسة تكوين العقد الجذرية بواسطة بكتيريا العقد الجذرية في منطقة الخليج الاهتمام الكافي من قبل الباحثين بالرغم من اهمية ذلك في زيادة انتاج المحاصيل البقولية . كما وانه لم تجر ابحاث في هذا المجال في سلطنة عُمان . لقد اجري هذا البحث لدراسة إمكانية بكتيريا العقد الجذرية المتواجدة في التربة العُمانية في تكوين عقد جذرية في نبات البرسيم ومقارنتها ببكتيريا عقد جذرية معروفة بالكفاءة العالية والاداء المتاز التي استوردت من قسم تثبيت النيتروجين للنباتات البقولية في المناطق الاستوائية - جامعة هاواي - الولايات المتحدة الأمريكية . لقد اثبتت الدراسة مقدره بكتيريا العقد الجذرية التي تم عزلها من مناطق مختلفة من سلطنة عُمان والمستوطنة في التربة العُمانية على تكوين عقد جذرية في نبات البرسيم وتثبيت النيتروجين الجوي وزيادة الانتاج إلا ان البكتيريا المستوردة من جامعة هاواي وعلى الأخص البكتيريا (TAL1373) تميزت عليها من حيث الفاعلية والأداء ، كما انها استطاعت التأقلم والتكيف في البيئة العُمانية ومنافسة بكتيريا العقد الجذرية المتواجدة في التربة .

ABSTRACT: Three native *Rhizobium* strains were isolated from Musandam, Buraimi and the Agricultural Experiment Station at Sultan Qaboos University in the Sultanate of Oman. An internationally known *Rhizobium* strain (TAL1373) was obtained from the Nitrogen Fixation for Tropical Agriculture Legume Collection at the University of Hawaii, U.S.A., for comparison. The different abilities of the strains to nodulate *Medicago sativa* L., were investigated. Different plant parameters, namely, the fresh and dry weights of the shoot (g/plant), the number of nodules per plant and the nitrogenase activity (μ mole C_2H_4/sample/3 hours) were measured. The results showed that the native rhizobia were capable of nodulating *M. sativa*, but inoculation with effective known strains such as TAL1373 enhanced nodulation.

The worldwide distribution of *Medicago sativa* (lucerne or alfalfa) is attributed to its well-known characteristics, longevity, symbiotic nitrogen fixation, high yield of good quality herbage and adaptability to different climatic and soil conditions (Maurice, 1985). *Medicago sativa* is one of the most important fodder crops in the Sultanate of Oman and it is a major source of income for many families. Four different varieties, viz. Al-Omani, Al-Batini, Al-Quriati and Al-Dhofari, are grown.

To our knowledge, no research work on the nodulation of *M. sativa* by *Rhizobium* has been conducted in the Sultanate of Oman. This experiment investigates the abilities of some native *Rhizobium* isolates to effectively nodulate *M. sativa* and to compare their performance with an internationally known isolate.

Materials and Methods

Three native *Rhizobium* isolates were obtained from different areas (Musandam, Buraimi and the SQU Agricultural Experiment Station) where *M. sativa* is grown and an internationally known isolate TAL1373 was obtained from the Nitrogen Fixation for Tropical Agriculture Legume Collection at the University of Hawaii, U.S.A. NIFTAL Project. All isolates were grown in 11 fermenters containing 250 cm^3 yeast mannitol broth. The inoculated fermenters were placed inside a shaker incubator set at 25 C and the number of bacterial cells cm^{-3} counted 5-7 days later using the drop plate method of Hoben and Somasegaran (1982). When the concentration reached 10^9 cell cm^3, the inoculum was used for seed inoculation of a local variety known as Al-Batini. The inoculant was prepared in which finely ground charcoal was used as a carrier and a solution of 40% gum arabic was used as a sticker.

The inoculated seeds were sown in pots containing soil that was autoclaved at 121 C for 2 hours at 15 $lb.in^{-2}$. Three local isolates and an imported one were used. Two controls were made. Control 1 was without an inoculant or urea while Control 2 contained 40% urea only (25 kg N /ha). Ten pots for each treatment were made, placed in

TABLE 1

Plant parameters in Medicago sativa controls and experiments inoculated with five different varieties of Rhizobium. n = number of plants; values are mean ± standard deviation; means denote with same letters are not significantly different, Tukey test (P<0.05).

	Fresh weight-shoot (g/plant)	Dry weight-shoot (g/plant)	Number of nodules per plant	Nitrogenase activity (um moles C_2H_4/Sample/3 hours)
Number	70	70	70	2
Control 1	1.39 ± 0.49**bc**	0.25 ± 0.09**bc**	7.4 ± 0.7**d**	2.53
Control 2	1.10 ± 0.66**c**	0.18 ± 0.12**c**	1.7 ± 0.4**e**	2.40
TAL 1373	2.23 ± 1.00**ab**	0.42 ± 0.17**ab**	39.4 ± 1.5**c**	15.79
SQU Farm	2.34 ± 0.92**a**	0.47 ± 0.19**a**	26.7 ± 1.0**a**	25.67
Buraimi	2.01 ± 0.81**ab**	2.01 ± 0.81**ab**	24.6 ± 0.7**a**	10.69
Musandam	2.06 ± 0.97**ab**	2.06 ± 0.97**ab**	19.2 ± 0.9**ab**	25.39

the greenhouse, and were watered daily. Each pot contained more than ten plants. After 30 days of growth, one pot from each treatment was used and 10 plants were randomly removed. The following parameters were measured weekly for seven weeks: the fresh and dry weights of the shoot; in which the shoots were placed in an oven at 75°C for three days until a constant weight was obtained; and the number of the nodules were counted. Nitrogenase activity was measured by acetylene reduction using a gas chromatograph in which ethylene concentration was measured in 1 ml samples of vacutainer headspaces, taken using plastic disposable syringes, with a Hewlett Packard Gas Chromatograph (5580 A poropak N Column 80-100 mesh 2 m length) equipped with a flame ionization detector. A column temperature of 80°C with a carrier gas flow rate of 50 ml min^{-1} was used. The gas chromatograph was calibrated using standard ethylene gas samples and peak areas were integrated using a Hewett Packard Integrator. Each vacutainer plus sample was weighed, dried and reweighed, and the amount of ethylene produced normalized by sample dry weights. The *u*mole C_2H_4/plant/hour was calculated according to Ciat (1988).

Results and Discussion

The results of the measurements of the different plant parameters namely, the fresh and dry weights of shoot (g/plant), the number of nodules per plant and the nitrogenase activity (*u*m mole C_2H_4/sample/3 hours) are shown in Table 1.

When the fresh and the dry weights of the plants were analyzed, statistically significant differences were found ($P<0.05$). SQU Agricultural Station strain was not significantly different from Buraimi, Musandam and TAL 1373 strains, but it was significantly different from Controls 1 and 2. TAL 1373, Musandam, Buraimi and Control 1 were not significantly different from each other, but they were different from Control 2. Control 1 and 2 were not significantly different from each other. The local strains were comparable to TAL 1373.

However, SQU Agricultural Station strain was found to be better than Control 1, whereas Buraimi, Musandam and TAL 1373 were the same as Control 1. This was expected because the SQU strain was originally isolated from the same soil where control 1 and 2 were grown..

When the number of nodules were analyzed for all the treatments, TAL 1373 was found to produce the highest number of nodules and was significantly different from all other strains and the controls. The three native strains were not significantly different from each other, but produced significantly more nodules than Controls 1 and 2. Control 1 produced more nodules than Control 2. The least number of nodules produced by Control 2 is attributed to inhibition by urea. Dogra & Dudeja (1993) found that nitrogen fertilizer affects nodulation by limiting the attachment of rhizobia to the root hair, decreasing lectin binding sites, and decreasing infection thread formation. Furthermore, they found that nitrogen fixation is inhibited by nitrogen fertilizer even if nodules are formed. Although many legume species have direct access to biologically fixed nitrogen, they prefer to utilize the available nitrogen in the soil rather than develop a root nodule symbiosis with *Rhizobium*. It appears that TAL 1373 has competed successfully with native rhizobia and produced the highest number of nodules.

The highest nitrogenase activity was recorded for strains from the SQU Agricultural Station and Musandam. TAL 1373 and Buraimi showed intermediate level of activity, while the lowest activity was shown by the Controls. Somasegaran and Hoben (1994) reported a positive relationship between shoot and nodule dry weights. In this study, we found a positive relationship between the number of nodules and the fresh weights of

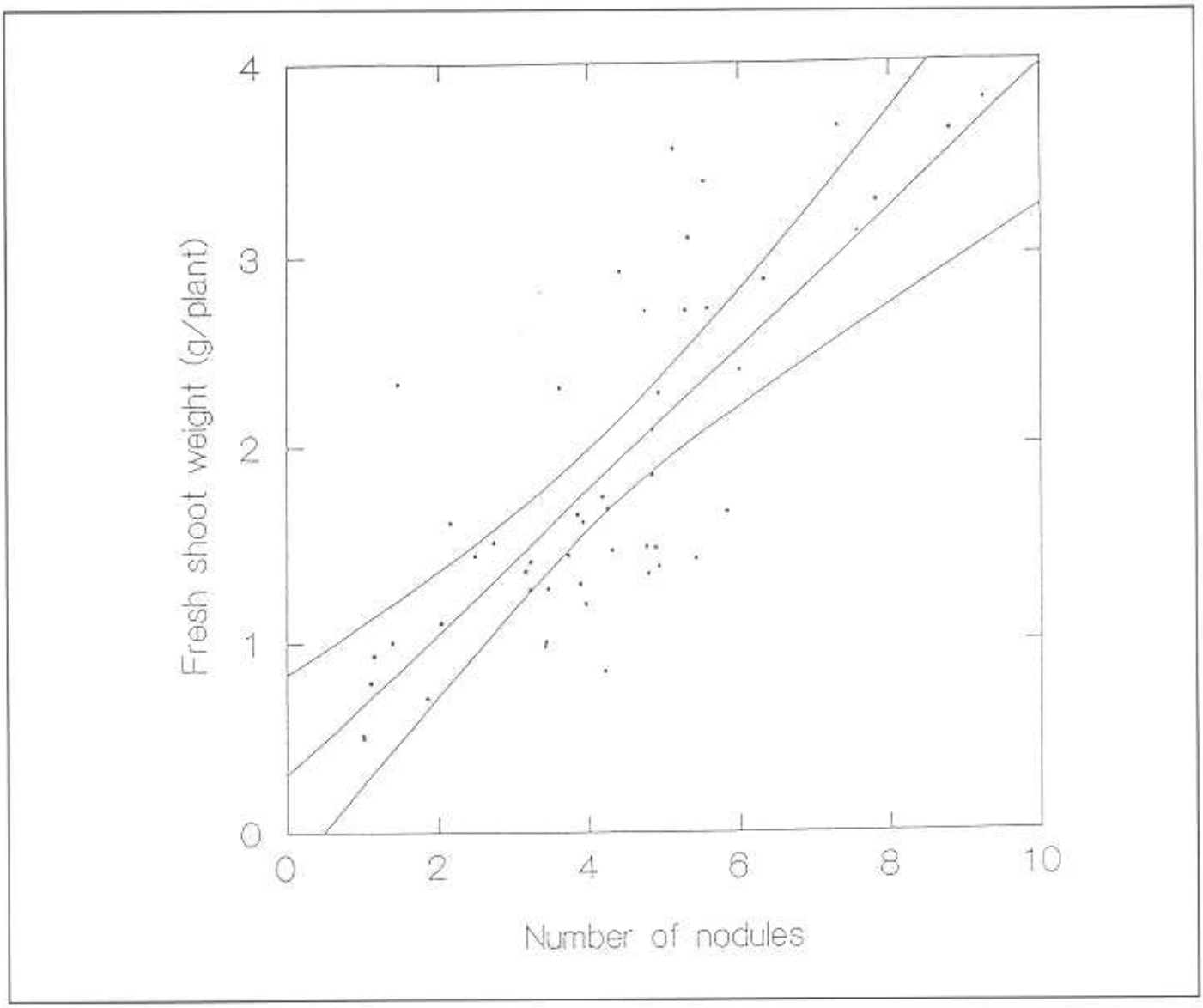

Figure 1. The relationship between the number of the nodules and the fresh weight. The inner regression line fitted with the model Y = a+ b X and 99%.

the shoot of the plant (Fig. 1). The equation describing this relationship is: fresh shoot weight = 0.307 + 0.387 (number of nodules). The ANOVA on regression rejected the null hypothesis, $H_0 = b = 0$ indicating a significant relationship ($F = 61.225$; $P < 0.0001$). The correlation between these two factors was also significant ($r = 0.714$, $P < 0.001$). From the results, we conclude that the Omani soil contains rhizobia that were able to fix atmospheric nitrogen. However, inoculation of seeds with good strains of rhizobia will enhance nodulation, and increase fresh and dry weights of plants. According to Jardim Freire (1984), the success in obtaining high rates of nitrogen fixation by *Rhizobium* - legume symbiosis - depends on many factors including the effectiveness and efficiency of *Rhizobium* strains present in the soil or inoculum in relation to legume species or varieties and the competitive ability of the introduced rhizobia against native rhizobial population, TAL 1373, tested in this arid part of the world for the first time, was able to adapt to the prevailing soil and climatic conditions and to compete with the native rhizobial population.

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