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Effects of Two Different *Rhizobium* Strains on Nodulation and Growth of Lucerne (*Medicago sativa* L.) in an Acid Soil

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菌系를 달리하는 根瘤菌이 酸性土壤 條件에서 알팔파의 根瘤形成과 生長에 미치는 効果

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ABSTRACT

To evaluate *Rhizobium meliloti* BALSAC, a strain selected from Canada as an acid tolerant one, and ordinary lucerne inoculant in acid condition, lucerne (*Medicago sativa* L. cv. Wairau) was inoculated and/or pelleted in the laboratory, and grown for two months in an acid soil (Lismore silt loam, pH 5.4) with three levels of lime in the glasshouse. The results of controlled (non-inoculated), nitrogen fertilized, laboratorial and commercial inoculated seeds were compared to give the following conclusions:

1. There was no significant difference in the top and root dry matter yields between two *Rhizobium* strains. However, Balsac inoculant showed higher single nodule dry matter weight and relatively higher number of larger nodules than the ordinary inoculant.
2. Lime application increased dry matter yields of plants and nodules, and the number of nodules per pot and the increase of nodules on the lateral roots in both inoculants. Lime application also caused an evenly distribution of nodules on the root by showing an increase of nodules mainly on the lateral roots.
3. Fertilizer nitrogen without inoculant slightly increased the nodulation percentage, the nodule dry matter weight per nodule and the relative proportion of larger nodules.
4. Commercially inoculated and pelleted seed showed less consistent results.
5. Relatively larger variations in measuring nodule characteristics was discussed and concluded that extreme cares should be given to reduce the variation.

INTRODUCTION

Lucerne is well known to be particularly sensitive to acid soils even though it has a great deal of agronomic advantages in terms of higher yield as feed and drought resistance once it is established on dry hill country.

One possible approach to overcoming the problems of establishing lucerne on acid soils is to modify the soil environmental conditions to suit the plant, and the other is to select strains of *Rhizobium meliloti* which is more tolerant of acid soils. The latter approach may have distinct possibilities when heavy liming is not economic in hill or high country owing to high transport cost (White, 1966). So far, however, relatively little information on this approach has been available.

In relation to the latter approach a new strain of *Rhizobium meliloti* was selected in Canada and named *R. meliloti* BALSAC (Bordeleau pers. comm.). This inoculant is now commercially available. The purpose of this paper is to compare this new *Rhizobium* strain, called Balsac here, with an ordinary strain in acid soil conditions.

MATERIALS AND METHODS

Cultivar: Lucerne (*Medicago sativa* L. c.v. Wairau) was used in this experiment.

Soil Treatment: Lismore silt loam (pH 5.4) was used. The soil of top 0 - 15 cm was sampled, mixed, and sieved through 10 mm mesh for potting. Soil pH was adjusted from pH 5.4 of nontreated soil to pHs 5.9 and 6.4 by liming equivalantly 400 and 1,020 kg ha^{-1} as $Ca(OH)_2$. For basal dressings 100 unit of P as calcium phosphate, 50 unit of S and K as potassium sulphate, and 0.2 kg ha^{-1} sodium molybdate were applied. Lime and other fertilizers were mixed through the soil before potting.

For treatment No. 7, 50 unit of N as urea was applied, in which 25 unit of N was applied at sowing time and the other half at 30 days later. The ordinary inoculant branded "Rhizocote" was supplied by the Coated Seed Ltd. and *Rhizobium meliloti* BALSAC, was supplied by the Fruit Growers Chemical Co. Ltd., Nelson, New Zealand. Pots of which diameter was 15 cm were filled with 1800 g of air-dried soil.

Sowing and Watering: Twenty seeds per pot were sown at 1.0 cm depth. After sowing, newspaper was placed on the top of pots in order to prevent dessication of the pot soil until seedling emergence. Three weeks later from sowing, plants were thinned to 10 per pot. All pots were weighed and watered with demineralized water to maintain 80% of field capacity.

Growth Period and Growth Condition: Plants were grown for 9 weeks. To prevent excessive

Seed Treatment: The following seed treatments were given;

Treatment No.	Descriptions
0	Control, Neither inoculated nor pelleted
1	Inoculated with peat slurry, ordinary *Rhizobium*
2	Inoculated with peat slurry, Balsac *R.*
3	Inoculated and pelleted, ordinary *R.*
4	Inoculated and pelleted, Balsac *R.*
5	Commercial inoculated and pelleted, ordinary *R.*
6	Commercial inoculated and pelleted, Balsac *R.*
7	Neither inoculated nor pelleted but Nitrogen fertilized only.

heat during the day time, a cloth shelter was covered on the top of the glasshouse. During the growth period, the minimum temperature was varied from 11 to 17°C, averaged 12.4°C and the maximum temperature was varied from 15 to 30°C, averaged 22.7°C.

Experimental Design: A randomized block design with 6 replicates was applied to 144 pots (1 cultivar, 3 lime levels, 8 seed treatments and 6 replicates). Statistical analysis of data was done per pot basis.

Harvesting and Data Collection: Due to the time elapse during harvesting work, plants within a replicate were treated in a same sequence. To prevent loss of nodules and lateral roots, each pot was immersed in the water for 30 minutes and then root soils were carefully removed through the tap water. The following procedures were taken for measuring chraracters on single plant basis:

Step I, Separation of nodulated and unnodulated plants:

Step II, Observation of nodule status such as nodule numbers, nodule weight, nodule size, vertical distribution of nodules, and nodule position.

Step III, Separation of top and root and drying for DM yields.

Five categories were based on the size were used for grading individual nodules, as shown below;

Grade	Description of nodule
A	Very large, over 5 mm in length, clustered, pink,
B	Medium, 3-5 mm in length, clustered, pink
C	Intermediate, 2-3 mm in length, rod shape.
D	Small, round, approx. 1 mm in diameter, no pigmentation.
E	Very small, ineffective in appearance, no pigmentation

For the analysis of vertical and positional distribution of nodules, nodules on tap roots and on lateral roots were separately counted at 3 cm intervals from the crown.

RESULTS

Top and root dry matter: The main effect means of dry matter yield and their effectiveness, which was shown by the values of each treatment to the values of fertilizer nitrogen pot, were given in Appendix I, and the effect of each individual treatment was shown in Fig. 1. At lower pH condition, there was a significant difference in dry matter yield between uninoculated seed and inoculated and/or pelleted seed, but there was no difference between two different *Rhizobium* strains. With the increase of lime, top and root dry matter yields were linearly increased. In in-

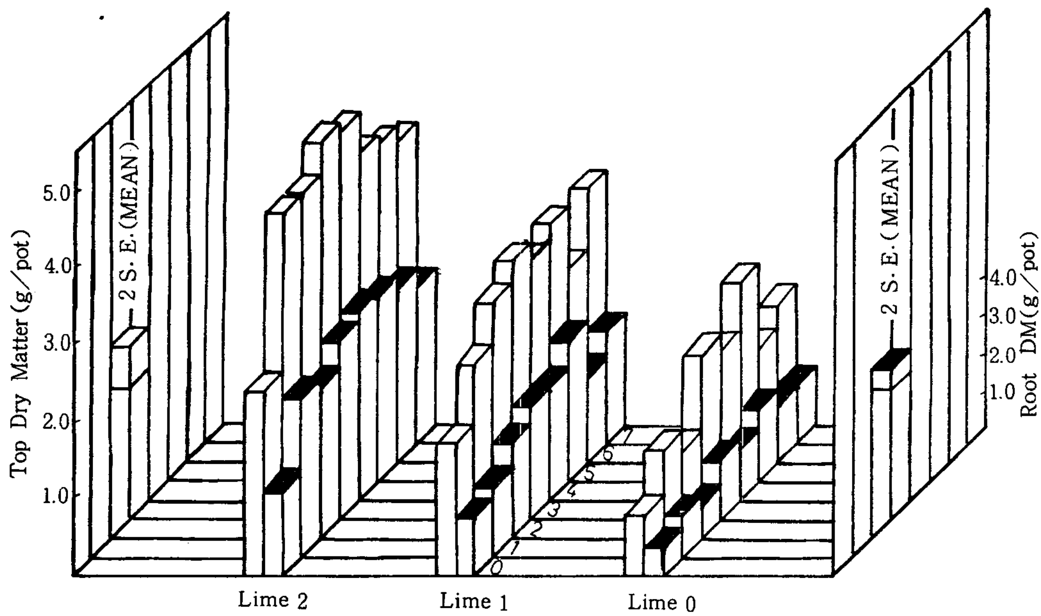


Fig. 1. Effect of lime and seed treatments on top and root yield.

oculated and pelleted seed, top and root dry matter yields were slightly increased comparing to those of inoculated seed, resulting an increase of the effectiveness of nodulation.

Nodulation percentage and nodule dry weight: Nodulation percentage and nodule dry weight (mg/pot) were shown in Fig. 2, and their main effect means were in Appendix table I. Without

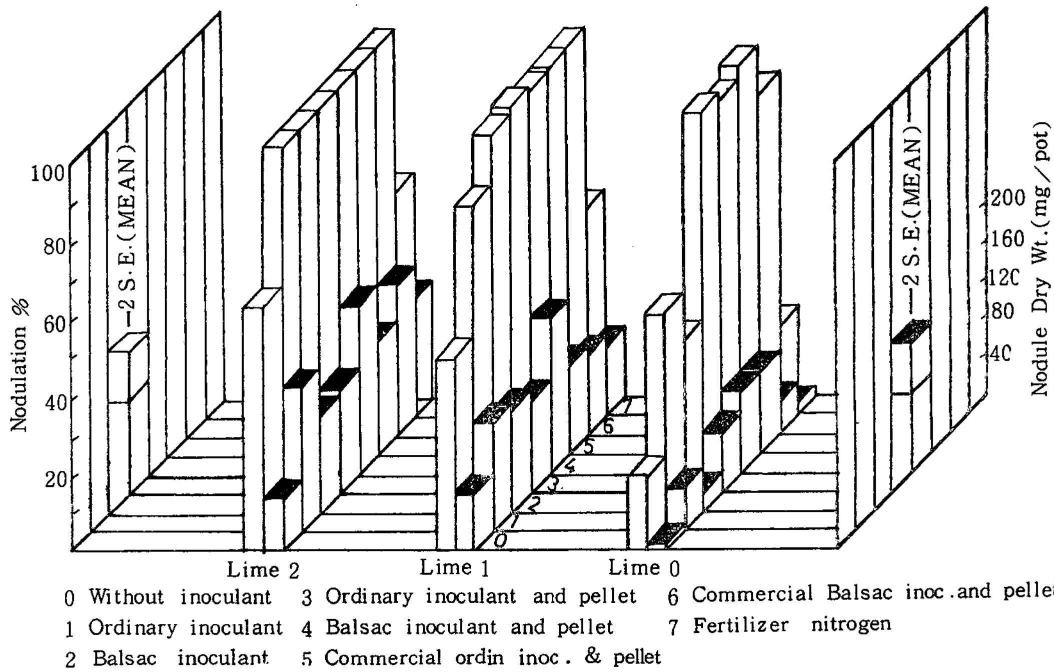
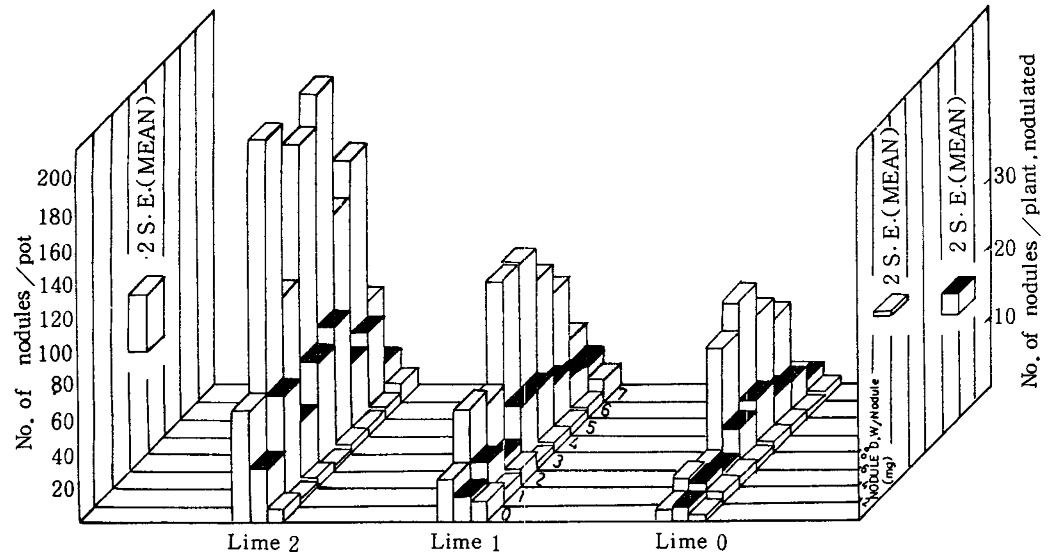


Fig. 2. Effect of lime and seed treatments on nodulation percentage and nodule dry weight.

liming, ordinary inoculant showed slightly higher nodulation percentage than Balsac inoculant but no significant difference. With the increase of lime, there was no difference in nodulation percentage either between two *Rhizobium* strains or between seed treatments.

Nodule dry weight of ordinary inoculant was consistently higher at all levels of lime, however, this trend was reversed when the seeds were inoculated and pelleted, showing consistently higher nodule dry weight in Balsac inoculant. Commercial inoculated and pelleted seeds showed no significant difference either in the nodulation percentage or in nodule dry matter weight between two *Rhizobium* strains.

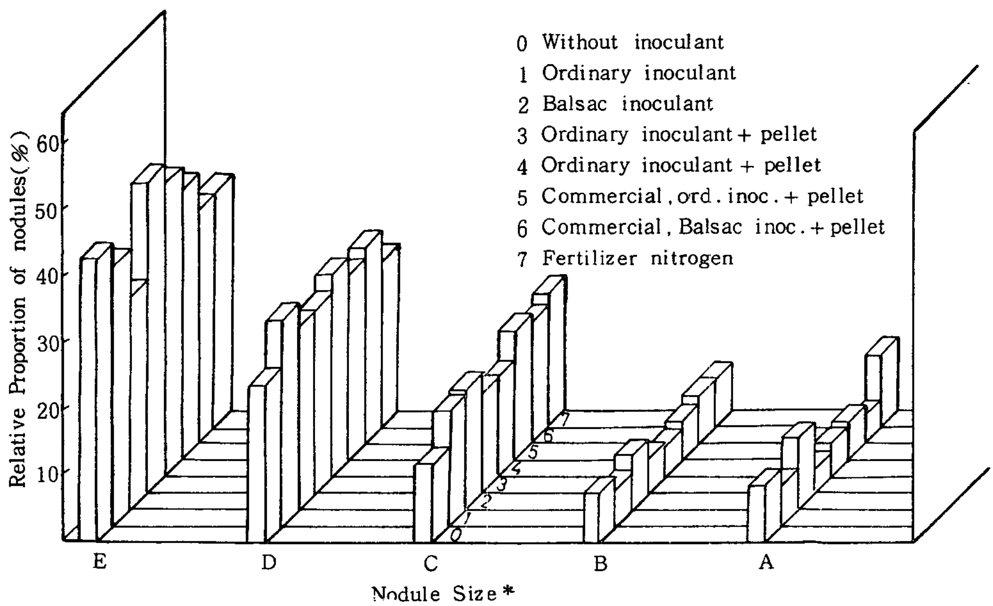
No. of nodules and nodule dry matter weight per nodule: No. of nodules per pot and per nodulated plant and nodule dry matter weight per nodule were shown in Fig. 3, and their main effect means in Appendix table II and I. Appearantly, ordinary inoculant showed more nodules than Balsac inoculant when the seeds were inoculated only. The difference in nodule number per pot between two *Rhizobium* strains was enlarged by increasing lime levels. Again, this trend was reversed when the seed was inoculated and pelleted even though the difference was not significant. With the increase of lime, the nodule numbers were linearly increased. Commercial inoculated and pelleted seed showed no significant difference in the no. of nodules between two *Rhizobium* strains. No. of nodules per nodulated plant showed relatively the same difference between the treatments as the number of nodules per pot. On the other hand, nodule dry matter weight per nodule was adversely effected by the seed treatments, i.e.



0 Without inoculant 3 Ordinary inoculant and pellet 6 Commercial Balsac inoculant and pellet
1 Ordinary inoculant 4 Balsac inoculant and pellet
2 Balsac inoculant 5 Commercial ordinary inoculant and pellet 7 Fertilizer nitrogen

Fig. 3. Effect of lime and seed treatments on no. of nodules per pot and nodulated plant and nodule dry weight per nodule.

single nodule weight with inoculant was lower than nitrogen fertilized pot and/or controlled pot. In terms of single nodule dry matter weight there was not any consistency in the difference between two *Rhizobium* strains. But Balsac inoculated and pelleted seed showed slightly higher single nodule



*From A, very large, over 5 ㎜ in length, to E, small, ineffective in appearance, no pigmentation

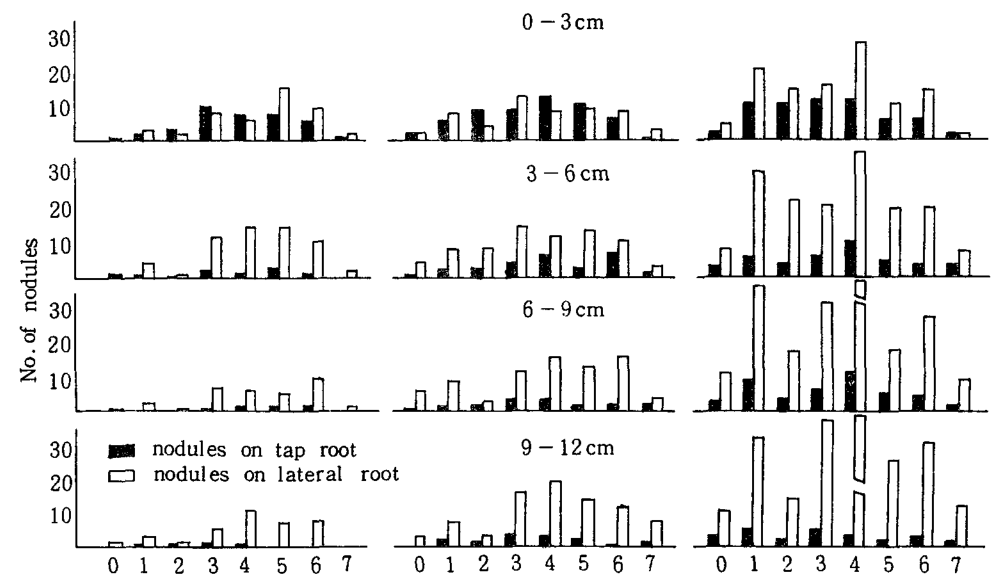
Fig. 4. Effect of seed treatments on nodule size distribution shown in relative proportional percentage in which lime effect was compounded.

dry matter weight in average.

Lime application showed a quadratic effect on single nodule dry matter weight.

Nodule size distribution: Distribution of single nodule size which was shown in the relative percentages based on the five categories (from A, the largest, to E, the smallest and ineffective in appearance) was shown in Fig. 4, and the main effect means were summarized in Appendix table II. Balsac inoculant produced proportionally higher number of larger nodules (Grade A and B) than ordinary inoculant, while there was not any consistent changes in the smaller nodules between two *Rhizobium* strains. Lime application linearly increased the number of smaller nodules. Commercial inoculated and pelleted seed showed less insistent results.

Vertical and positional distribution of nodules: As shown in Fig. 5 and Appendix table II and III, there was not any significant difference between



0 Without inoculant
1 Ordinary inoculant
2 Balsac inoculant
3 Ord inoc. + pellet
4 Balsac inoc. + pellet
5 Ord. inoc. + pellet commercial
6 Balsac inoc + pellet commercial
7 Fertilizer nitrogen

Fig. 5. Effect of lime and seed treatments on vertical distribution of nodules on tap and lateral roots.

two *Rhizobium* strains. However, in terms of relative number of nodules which was shown as a proportion to the total nodules per pot, Balsac inoculant showed proportionally higher number of nodules in the upper part of the root. This proportion was reversed in the position downward of the root (i.e. 12 to 18 cm from the crown). Lime application increased the number of nodules at all the vertical distances (Appendix table III). When the nodules were classified into two different positions as nodules on tap roots and nodules on the lateral roots, there was not consistent difference between two *Rhizobium* strains. Nodules on the tap roots were rapidly decreased as the vertical point was positioned downward, however, the decrease was retarded as the lime increased.

Nodules on the lateral roots were not decreased as the vertical position was downward, but moderately increased without lime. The decrease was retarded as the lime increased. Commercial inoculated and pelleted seed showed less nodules on both tap and lateral roots than laboratory inoculated and/or pelleted seed as a whole.

DISCUSSION

Many researches have been concentrated on the selection of symbiotically superior strains of nodule bacteria, and some of them have been successful in Australia and New Zealand. In Australia, strains used in inoculants in the early days are now regarded as being of only intermediate effectiveness (Bergersen 1970) and in New Zealand such an improvement enables to establish legumes with inoculant only quite successfully on hill country (Musgrave, pers. com.) From this experiment the Balsac inoculant was not superior to the ordinary inoculant in terms of dry matter yield but Balsac showed some indications of better potency in terms of nodule characteristics such as nodule numbers, single nodule weight, nodule size distribution, and nodule position. As a whole, Balsac inoculant produced less nodules, slightly higher single nodule weight, proportionally higher number of larger nodules particularly near the crown. These are implicating the higher effectiveness of the inoculant in gerneral.

As described by Thompson et al. (1964), however, there was a large variation in the nodule status as shown in Appendix tables I, II, and III. This finding has an important implication to any practical research with inoculants bearing two problems which could be associated with the successful evaluation of *Rhizobium* strains.

Firstly, the higher degree of variation shown as coefficient of variation, suggests that a large number of samples (plants) should be used in an experiment in order to make a decision with a higher confidence.

Secondly, there might be a higher degree of interactions among host variety x bacterial strain x environment. In relation to this problem, Gibson (1962) studied the effectiveness of the nodulation of 15 varieties of lucerne with single strain of *Rhizobium meliloti* and found that highly significant host variety x bacterial strain interactions. This indicates that the generalizations regarding varieties and bacterial strains should be interpreted with highly caution. Bearing these factors in mind, the number of plants, observed in this experiment, was at least 60 plants for each treatment. This seems to be sufficient for the measurement of the nodule characteristics.

But in the practical point of view, it takes a lot of time for measuring nodule characteristics and thus the time elapse during the observation may cause a systematic error in the experiment. This fear should be reduced by introducing a new technique on this matter of science.

The reversed effects of pelleting on the nodule characters such as nodule dry matter weight and nodule numbers are not clear but seems partly to be attributed to the response of inoculants and partly to the changes of micro-environmental condition due to pelleting. Further investigation is required for any critical evaluation of the effects.

From this experiment, the seed treatments are considered to be helpful to evaluate inoculants but there expected some degrees of complication in interpreting the results. Commercially inoculated and pelleted seed used in this experiment failed to show any consistent result and, as a whole, it was inferior to laboratory-made fresh inoculants. Therefore, extreme care should be given to the use of commercially inoculated and pelleted seed for the inoculant evaluation. Fertilizer nitrogen acted positively for some nodule characters by increasing nodulation percentage, nodule size, and top and root dry matter yields throughout the experiment. This findings were supported by Bergersen (1960) who insisted that the use of strategic amounts of fertilizer nitrogen at the beginning of the growing season may be necessary for the improvement of the symbiotic properties.

There was a clear cut response of lime to plant and nodule characters in the experiment. This response seems mainly due to the acidity of the soil used. From this experimental result, it would be sufficient to suggest that the most of lime response by lucerne could be attributed to the improvement of nodulation of lucerne on the moderately acid soils as suggested by Munns (1965, and 1968), Date (1970), White (1970), Janson and White (1971) and Choe et al. (1979). Among the lime responses, particular interest came to the increase of number of nodules and the vertical and positional distribution of the nodules as shown in Fig. 3 and Fig. 5. With the increase of the amount of lime, there was a rapid increase of nodules in both inoculants. With the farther analysis, it was found that the increase of nodules was attributed to the increased nodules on the lateral roots, and that the nodules were well evenly distributed on the whole root mass, while there was a rapid decrease of nodules apart from the crown without lime as shown in Fig. 5.

It may not be sufficient to evaluate inoculants with two months of growth period considering the persistency of the bacteria, however, some evidence of the effectiveness of *Rhizobium meliloti* BALSAC are recognized from this experiment; higher relative proportion of larger nodules in the lower pH condition.

要 約

酸性土壤에 適應할 수 있는 系統으로 캐나다에서 育成普及된 알팔파 根瘤菌 *Rhizobium meliloti* BALSAC과 一般的으로 알팔파 栽培用으로 使用되고 있는 보통 根瘤菌이 알팔파 뿌리혹 形成과 生長에 미치는 效果를 比較하기 위하여 pH 5.4 程度의 酸性土壤을 石灰施用으로 pH를 5.9, 6.4로 교정한 뒤 몇가지 種子處理 즉 無處理, 無處理+N施用, 根瘤菌接種, 根瘤菌 接種+石灰被覆으로써 硝子室에서 2個月間 포트試驗하여 얻은 結果를 要約하면,

1. 알팔파의 乾物量 生產에는 두 根瘤菌系統間에 差異가 없었으나 뿌리혹 形成에 있어서는 Balsac 系統의 效果가 認定되었다.
2. 石灰施用으로써 植物體와 뿌리혹의 乾物重 및 뿌리혹數가 增加되었으며 뿌리혹의 增加는 주로 側生根에서의 뿌리혹數 增加에 基因하였다.
3. 根瘤菌을 接種하지 않고 窒素肥料만을 施用함으로써 뿌리혹을 形成한 個體比率, 뿌리혹의 乾物重 및 全體뿌리혹數에 대한 굵은 뿌리혹의 相對的 比率이 增加되었다.
4. 商業的으로 生產된 根瘤菌의 種子處理 效果는 一定한 傾向을 나타내지 못하였다.
5. 뿌리혹과 關聯된 形質調査에서는 相對的으로 그 變異係數가 컸으며 그 變異를 減少시키고 推定值에 대한 信賴度를 높이기 위해서는 特別한 考慮가 있어야 할 것으로 보였다.

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Appendix I. Main effect means of top and root D. M., top/root, nodulation percentage, no. of nodules per nodulated plant and nodule D. M. per pot and per nodule.

Treatment	Top D. M. (g/pot)	Effectiveness*	Root D. M. (g/pot)	Effectiveness*	Top/Root ratio	Nodulation (%)	Nodule (mg/pot)	D. M. mg/nodule	No. of Nodules/ plant, nodulated
Without inoculant	1.663	0.54	1.513	0.55	1.15	45.7	41.2	1.68	4.7
Ordinary inoculant	2.832	0.92	2.404	0.88	1.24	83.5	103.3	1.56	8.1
Balsac inoculant	2.978	0.97	2.484	0.91	1.28	80.5	76.8	1.56	5.5
Ord. inoc. +pelle+	3.541	1.16	3.084	1.13	1.25	99.5	89.4	0.87	10.4
Balsac inoc. +pelle+	3.373	1.10	3.002	1.10	1.23	98.3	139.3	1.24	11.8
Commercial									
Ord. inoc. +pelle+	3.472	1.33	3.688	1.35	1.00	100.0	98.1	1.23	8.2
Balsac inoc +pelle+	2.782	0.91	2.798	1.02	1.06	95.9	97.9	1.22	8.2
Fertilizer nitrogen	3.064	1.00	2.735	1.00	1.16	56.3	66.9	2.05	4.9
Without Lime	1.730	0.56	1.410	0.52	1.28	65.6	46.0	1.20	4.6
Lime 1	2.933	0.96	2.641	0.97	1.16	87.9	97.7	1.77	6.4
Lime 2	4.227	1.38	4.090	1.50	1.07	93.9	124.3	1.26	12.2
S. E. (Mean)	0.273	–	0.285	–	0.077	6.14	13.82	0.361	1.53
C. V. (%)	22.5	–	25.7	–	16.03	18.2	38.0	63.1	48.3

* Effectiveness of nodulation = Values of each treatment / values of N control

Appendix II. Main effect means of no. of nodules, nodule size and vertical distribution of nodules.

Treatment	No. of nodules per pot	Nodule size distribution					Vertical distribution of nodules, cm					
		A	B	C	D	E	0–3	3–6	6–9	9–12	12–15	15–18
Without inoculant	33.4	2.9	2.5	4.9	8.8	14.3	3.6	6.1	7.8	6.3	5.5	4.1
Ordinary inoculant	92.3	5.9	5.3	16.1	28.8	36.2	15.2	17.6	19.6	16.0	13.8	10.1
Balsac inoculant	59.2	6.4	4.9	12.4	16.4	19.1	13.8	13.0	9.1	7.9	7.3	8.1
Ord. inoc. +pelle+	124.7	4.9	6.8	19.4	35.2	58.4	21.9	20.1	21.0	22.7	24.4	14.6
Balsac inoc +pelle+	142.2	7.2	7.0	21.3	43.4	63.3	24.8	27.4	28.6	29.5	22.4	9.5
Commercial												
Ord. inoc. +pelle+	97.6	5.7	5.9	19.2	27.0	39.8	19.2	19.6	15.4	17.0	16.2	10.2
Balsac inoc +pelle+	95.1	6.2	7.1	17.7	30.7	33.4	16.3	18.1	20.8	18.1	13.6	8.2
Fertilizer nitrogen	37.2	4.1	2.8	7.6	9.4	13.3	3.2	5.5	6.2	7.7	8.6	6.0
Without Lime	45.5	4.0	3.7	7.8	11.1	18.9	10.2	8.6	4.9	5.5	7.9	8.4
Lime 1	70.4	7.0	6.1	14.0	19.8	23.5	13.4	13.1	12.6	11.9	11.1	8.3
Lime 2	139.7	5.4	6.1	22.7	44.0	61.5	20.9	26.0	30.6	29.6	22.9	9.7
S. E. (Mean)	17.41	1.28	1.36	2.41	5.19	12.12	3.03	3.01	3.53	4.32	5.14	5.17
C. V. (%)	50.1	5.78	62.9	39.9	50.9	87.6	50.2	46.4	53.9	67.6	90.3	121.9

* A, very large : B, medium; C, small／medium ; D, small, round shape ; E, small, ineffective in appearance, no pigmentation.

Appendix Ⅲ. Distribution of nodules measured at the different root position and different root depth* in different seed treatment and lime application.

Sudtreat-ment	No Lime								Lime 1								Lime 2							
	Tap Root				Lateral Root				Tap Root				Lateral Root				Tap Root				Lateral Root			
	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
Without inoculant	0.0	0.2	0.0	0.3	0.2	0.8	1.0	1.2	1.7	0.7	0.8	0.3	2.0	4.8	6.7	3.2	2.5	3.0	3.0	3.0	4.5	8.7	11.8	11.0
Ordinary inoculant	1.5	0.7	0.0	0.5	2.8	4.3	2.8	3.0	5.5	2.3	1.3	1.3	7.3	8.8	9.0	7.2	9.7	5.8	8.3	3.2	20.3	30.5	37.2	32.8
Balsac inoculant	3.2	0.3	0.0	0.5	1.8	1.0	1.0	1.8	8.5	2.7	1.5	1.3	4.3	9.0	3.0	3.7	10.3	3.7	3.8	1.8	14.8	22.3	17.8	14.5
Ord. Inoc. +pelle+	10.0	1.8	0.7	0.8	8.3	12.2	7.2	5.8	8.5	4.2	3.7	3.5	12.3	15.0	12.2	16.0	11.0	6.0	6.3	4.8	15.7	21.2	32.0	37.0
Balsac inoc +pelle+	7.7	1.0	1.3	0.8	6.0	14.5	6.5	10.7	12.2	6.7	3.3	3.0	8.2	12.2	16.3	19.5	11.5	10.3	11.3	3.0	28.8	37.7	43.8	51.5
Commercial																								
Ord. Inoc. +pelle+	7.3	2.8	1.3	0.8	15.8	14.8	5.7	7.7	10.0	2.7	1.8	2.2	9.0	14.3	13.5	13.7	5.3	4.2	5.7	1.2	10.0	20.0	18.3	25.5
Balsac inoc +pelle+	5.5	2.8	1.3	0.8	9.2	11.0	9.5	8.0	5.7	7.0	2.0	0.2	8.2	10.5	16.2	11.7	5.7	3.5	4.8	2.8	14.8	20.3	28.3	30.8
Fertilizer Nitrogen	0.5	0.0	0.0	0.5	1.7	1.3	1.3	0.8	0.7	0.7	2.2	0.7	3.0	3.5	4.3	7.5	1.8	3.0	1.3	1.3	1.8	8.0	9.3	12.2
mean	4.56	0.98	0.56	0.63	5.73	7.50	4.38	4.88	6.58	3.35	2.08	1.56	6.79	9.77	10.15	10.29	27.23	4.94	5.58	2.65	13.85	21.08	24.89	25.92
	S. E. of mean				C. V. (%)												S. E. of mean				C. V. (%)			
Tap root 3cm	1.73				69.4								Lateral root 3cm				2.46				68.5			
6	1.34				106.0								6				2.81				53.8			
9	1.37				122.5								9				3.09				57.0			
12	0.92				139.4								12				4.08				71.2			

* Nodules positioned below 12cm from the base were excluded from the this table, because of their natural uncertainty.