legumes inoculation Research in Vietnam

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1. Legumes production and potential of legume inoculation

Legumes are traditional crops cultivated in Vietnam and play an important role in Vietnam's agricultural system. Legumes group are very various and divided follow : Groundnut, soybean and other legumes (mungbean, black bean, red bean, white bean, lima beans green peas.... Legumes which have short – term growth, adopted ability appreciate with many kinds of cultivation are cropped all over provinces in Vietnam. There are 6 legume cultivated zones in the country, depended on the soil type, climatic condition and production tradition. The current status of legume production in Vietnam showed in table 1.

***Table 1**: Legume production in different cultivated zones (1998)*

No	Cultivated zones	Groundnut			Soybean			Other legumes		
		Area	Yield	Production	Area	Yield	Production	Area	Yield	Production
1.	North mountains and midland	42,2	10,3	43,6	54,6	8,6	46,4	27,8	6,7	17
2.	Red river delta	23,5	15,1	35,6	24,5	13,5	3,3	10,0	7,7	7,7
3.	North middle Vietnam	71,1	13,1	93,4	3,3	10,0	3,3	30,8	3,4	10,5
4.	Coastal area of South middle VN	28,9	13,0	37,5	3,6	15,3	5,5	14,4	8,0	11,5
5.	High land of Tay Nguyen	16,8	10,2	18,9	12	8,8	10,6	39,7	5,1	20,3
6.	Southeast VN	68,8	18,2	125,0	15,3	8,4	12,8	77,4	5,7	44,3
7.	Cuulong delta	16,3	19,6	32,0	14,5	20,5	29,7	21,4	15,5	33,2
8.	**Total**	**269**	**14,3**	**386,0**	**127,8**	**11,1**	**141,3**	**221,5**	**6,5**	**144,1**

Area in 1000ha   Yield in quintal/ha
Production 1000tonnes/year

The dates indicates that the production of soybean and groundnut the main legume crops growing in Vietnam has increased continuously in the past 5 years, caused by expanding the cultivated area and increasing the legume yield. However legume yields in Vietnam remain low in comparation to those in other Asian countries where soil and climatic conditions are similar. So in Vietnam the potential of legume production is very large. Vietnam Agricultural science Institute (VASI) has been nominated by the Vietnamese government as the coordinator of two projects on improving soybean and groundnut production in Vietnam during the period 2001-2005. Legume cultivation area will be expanded in the next 5 to 10 years and see in the table 2.

***Table 2* : Plan for legume production in Vietnam**

Year	Groundnut			Soybean		
	Area	Yield	Production	Area	Yield	Production
2001	250.000	15.0	375.00	130.000	12.5	187.5
2005	330.000	18.0	700.00	500.000	18	902.0
2010	-			700.000	-	-

Research , development and dissemination of advanced cultivation technology to achieve high legume yields and reduce production cost are the wish of all Vietnamese agricultural scientists and farmers. Legume inoculation is one of the ways to meet those challenges. The Research on rhizobium symbiosis has been carried out in Vietnam for more than 20 years but the inoculant's production is limited

2. Research of legume inoculation in Vietnam

2.1. Using ^{15}N – Technique in the selection of Bradyrhizobiial strains

10 Bradyrhizobial strains are used to test. The pot experiments were carried out in sterile sand –

soil at VASI. The biological activity of rhizobial strains is measured by gas – chromatography and ^{15}N Atom excess in plant by Emission – spectrometer. The results of reasearch are presented in the table 3, 4 and 5. They showed, that all strains have ability to nodulate on groundnut 1686. So the strains THA 201, and TL 3-1 have not only higest ARA (3.933-5.513) nmol $h^{-1}pot^{-1}$, better dry biomas and higher total plant nitrogen content. Groundnut 1686 (60 days after sowing) derived 18,2 – 24,7% of total plant nitrogen from N_2 – fixation of Bradyrhizobium.

***Table 3**: ARA activity of Bradyrhizobium Strains*

No	Treatments	ARA activity (nmol/ plant/ hour	Plant dry matter (g/pot)
1	MAR 377	1920.4	3,90
2	TAL 1000	247.8	3,18
3	TAL 236	3933.4	3,87
4	NC –92 (IC-7001)	672.7	3,60
5	IC-7029	1207.9	4,55
6	5a/ 70 (IC – 7017)	495.6	3,55
7	98	123.9	3,78
8	THA 201	5513.0	4,38
9	TL 6-2	997.8	4,17
10	TL 3-1	4970.2	4,97
11	Control (non-inoc)	0.0	2,37
	LSD 0,05	2663.6	0,77

***Table 4**: Plant nirogen content of inoculated groundnut 1686*

No	Treatment	Plant nitrogen content	
		(%)	(mg/pot)
1.	MAR 377	2,21	86,17
2.	TAL 1000	2,77	87,94
3.	TAL 236	2,35	01,06
4.	NC – 92 (IC – 7001)	2,63	94,68
5.	IC – 7029	2,02	91,93
6.	5a / 70 (IC - 7017	2,13	71,36

7.	98	2,41	90,97
8.	THA 201	2,07	89,01
9.	TL 6-2	2,24	93,33
10.	TL 3-1	2,07	102,8
11.	Control	2,18	66,12
	LSD	0,24	18,45

Table 5: *N_2-fixing activity of 5 Rhizobium strains*

No	Treatments	Plant Nitrogen (mg/pot)	Ration of ^{15}N enrichted and total plant nitrogen content (%)	N_2- fixing activity	
				(%)	(mg/pot)
1.	Control	51,67	0,285	-	18,2
2.	MAR 337	86,17	0,225	21,1	21,7
3.	TAL 1000	87,94	0,215	24,6	24,2
4.	NC - 92	94,68	0,212	25,6	24,6
5.	98	90,97	0,208	27,0	24,7
6.	THA 201	89,01	0,206	27,7	
	LSD 0,05	18,45			

2.2. Studying on the Rhizobial inoculant production

Carrier plays an important role in survive and biological activity conservation of shizobia in the preparation. So liquid and solid formulations of Rhizobial inoculant are studyed. Bacterium is growth in manitol years extract (YMB), G5 and G6 medium. For the solid formulation Bradyrhizobium biomas are injected in sterile peat packages. The inoculants are preserved for 6 months. The result of liquid formulation is presented in the table 6. The date showed that the survival of different rhizobium strains in liquid and solid formulation are different G5 & G6 medium can be used for some rhizobium strains, but not for all, it should be tested new medium for new trains.

***Table 6**: Survival of Bradyrhizobium in liquid and solid inoculant formulations*

Preservation Time	Rhizobium strains	Rhizobium densities (CFU/ g/ ml)				
		G5	G6	YMB	American peat	Vietnam Peat
0 h	132	5,00 x10^9	8,50 * 10^9	1,69 *10^9	1,20 *10^9	1,32*10^9
	133	2,43 * 10^9	5,48 * 10^9	8,60 *10^9	1,76 *10^9	1,75*10^9
	57	1,28 * 10^9	9,40 * 10^9	7,10*10^8	9,40*10^8	9,40 *10^8
2 weeks	132	6,20 * 10^9	2,12 * 10^9	1,80 * 10^9	9,20*10^9	1,00*10^9
	133	4,80 * 10^9	8,50 * 10^9	1,16 *10^9	3,04*10^9	1,40*10^9
	57	14,6 * 10^9	6,60 * 10^9	1,31 *10^9	3,70*10^8	8,70*10^8
1 month	132	5,60 * 10^9	1,41 * 10^9	1,41 *10^9	7,40*10^9	2,54*10^9
	133	2,59 * 10^9	1,11 * 10^9	1,53 *10^9	2,07*10^9	1,38*10^9
	57	9,8 * 10^8	2,32 * 10^9	9,30 *10^7	2,18*10^8	1,89*10^8
2 month	132	3,12 * 10^9	1,36 * 10^9	5,56*10^8	7,80*10^9	1,81*10^9
	133	2,80 * 10^9	-	7,80 *10^8	2,72*10^9	2,76*10^8
	57	8,10 * 10^8	7,80 * 10^6	6,70 *10^7	6,90*10^8	6,06*10^8
3 month	132	2,15 * 10^9	1,25 * 10^9	5,60*10^8	2,00*10^9	1,56*10^9
	133	8,75 * 10^7	-	1,78*10^8	4,80*10^9	1,32*10^8
	57	9,4 * 10^7	5,70 *10^5	3,87* 10^7	5,40 *10^8	1,00*10^8
6 month	132	1,39 * 10^8	1,38 *10^8	2,28 *10^8	3,00*10^8	1,33*10^8
	133	5,50 * 10^7	-	5,10*10^7	2,10*10^8	1,21*10^8
	57	2,26 * 10^6	8,40 *10^4	9,60 *10^5	3,60 *10^8	1,20 *10^8

In collaboration with the Radiative Centre of Atomic Technique Institute, the National Institute of Agricultural Science has studied 3 different method for carrier stelizing. Effects of irradiation in the carrier proccesing and survive of Rhizobium can be see in the tables 7 and 8. With the irradiation dosage of 25 KGy peat can used as steril carrier for the rhizobial inoculant (table 7). The density of Rhizobium japonicum in the peat carrier based irradiated at 30KGy after for months of storage in P.E bags were $3,7.10^9$/g, as compared with $1,05 \times 10^9$/g when sterilizing by dry hot gas and $1,15.10^9$/g sterilizing by saturated steam (table 8).

Table 7: *Effect of irradiative dosage on the sterilization of peat carrier*

Irradiative dosage (kGy)	Total aerobic x 1000/g	Total mold x 1000/g
0	200	+++
15	0,14	0,03
25	0,05	0,00
35	0,04	0,00
45	0,02	0,00

Table 8: *Effect of carrier sterilized method to surveying rate of bradyrhizobium japonicum in nitragin*

Carrier sterilized method	Survival of Bradyrhizobium in the peat carrier based inoculant in the storage of (10^8 CFU/g)				
	2 hour	1 month	2 month	3 month	6 month
Non – sterilize	2,5	24,0	0,1	0,1	-
Dry hot gas	27	26,0	25,0	20,0	10,5
Saturated steam	2,5	25,0	25,5	23,0	11,5
Gammar irradiation	2,4	40,0	45,0	47,0	37,0

Dry hot gas 165^0C in 4 hours
Saturated steam 1,5atm in 2 hours
Irradiative dosage 30 KGy

2.3. Field testing of Rhizobial inoculant in different localities

To determine the need for and potential benefits of rhizobial inoculation of legumes in the different regions of Vietnam, experiments were conducted in the some provinces over country. The main objective of the research on Rhizobial inoculant has been on the effect legume crop yield, return on investment and the nitrogen replacement by Rhizobial inoculant. The result of demonstration showed in the absence of inoculation, the farmer would have to supply the crop with substantial amounts of fertilizer N.

Table 9: *Effects of inoculant formulations on soybean in new cultivated acrisol at Cu Chi - Ho Chi Minh City*

Treatment	Nodule no./ Plant	Nodule wt/ plant (mg)	Shoot DM (t/ha)	Grain yield (t/ha)
1. Uninoculated, 0N	1	25	5.0	1.67
2. Uninoculated, 40N	1	6	6.1	1.81
3. Liquid inoculant G5	12	280	7.1	1.96
4. Liquid inoculant G6	20	401	8.4	2.12
5.Peat inoculant using G5	16	351	7.9	2.00
6. Peat inoculant using G6	21	414	7.5	2.18
7. Peat inoculant using YMB	21	353	8.1	2.24
LSD (P = 0.05)	3	36	0.8	0.20

Pichture1: Some cultivated zone where Rhizobial inoculant is tested

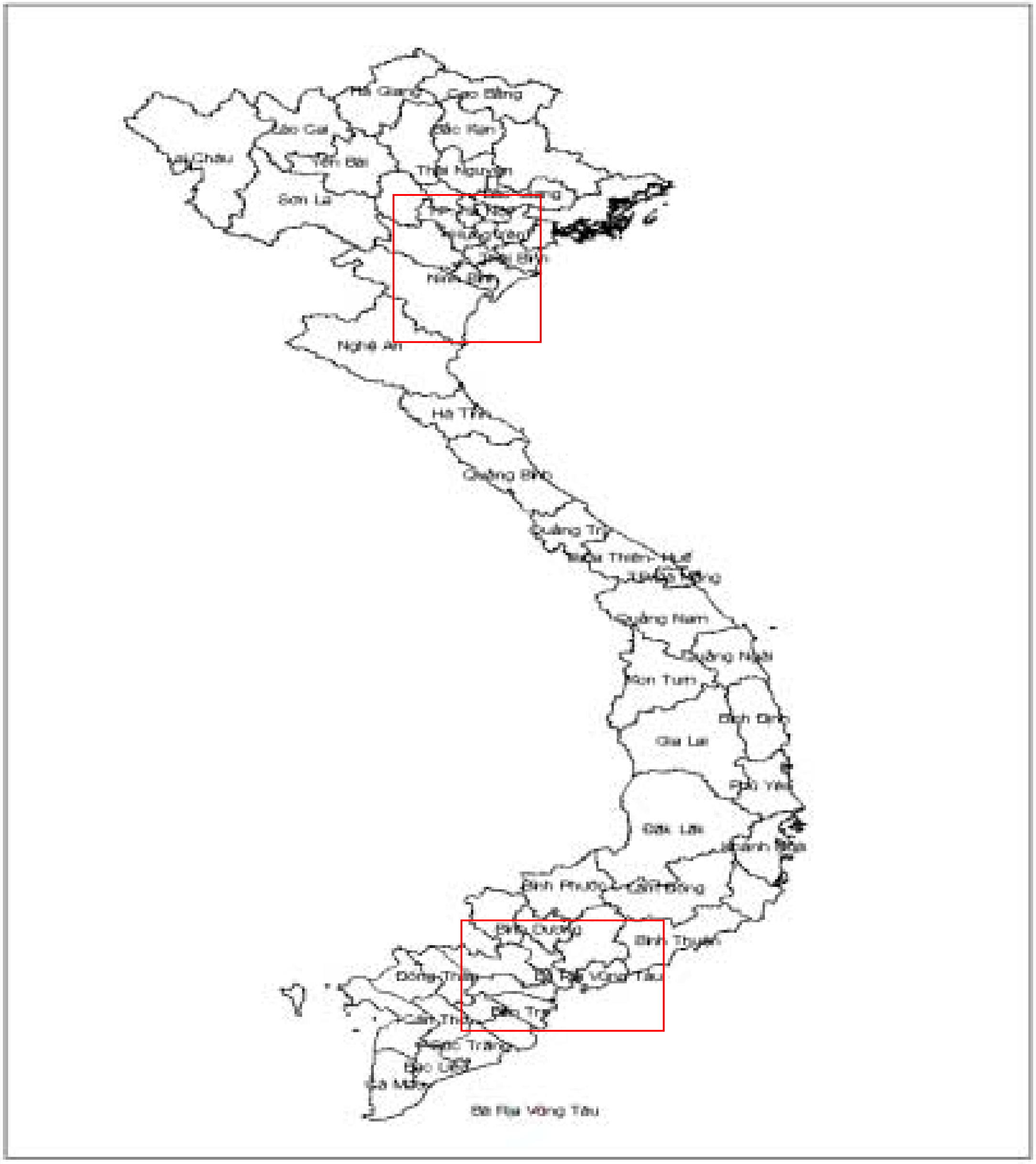

Table 10: *Effects of inoculant formulation on N and N_2 fixation of soybean on new cultivated acrisol at Cu Chi – Ho Chi Minh City*

Treatment	Shoot N (kg/ha)	Grain N (kg/ha)	% Ndfa	Crop N fixed[A] (kg/ha)
1. Uninoculated, 0N	124	71	18	33
2. Uninoculated, 40N	126	87	*	*
3. Liquid inoculant G5	209	103	35	110
4. Liquid inoculant G6	283	114	48	204
5.Peat inoculant using G5	276	111	41	170
6. Peat inoculant using G6	284	122	51	217
7. Peat inoculant using YMB	262	110	47	184
LSD (P = 0.05)	54	13	9	60

[A]crop N = shoot N x 1.5 to account for below – ground N

The table 9 and 10 showed Nodulation, shoot dry matters, grain yield % N dfa responded strongly to inoculation. Shoot DM increased of 42-68 %. Grain yield was improved by 17-34%. The % dfa values were 18% for unioculat plant and in 35-51% for the inoculated plants. The diffirences in shoot N contents and % Ndfa values between inoculated and uninoculated plots compounded to generate evn lager diffirences in crop N fixed. It was 33 Kg N/ ha for uninoculated and 110-217 Kg N/ha for the inoculated.

There were no difference beween the G5 &G6 formulations, no differences between liquid and peat-based inoculaion. So can use broth liquid and peat inoculaion for legume.

Table 11: *Inoculation and fertilizer N effects on soybean grown in a new soybean cultivated area of Dong Nai province*

Inoculation/ fertilizer N	Nodulation (no/ plant)	Nodulation (g fresh wt/ plant)	Shoot DM (t/ha)	Grain yield (t/ha)
+ inoc	19	1.44	7.85	1.20
- inoc	11	1.14	7.43	1.09
0N	11	0.78	5.68	0.70
20N	17	1.57	9.65	1.36
40N	17	1.51	9.27	1.37

Although this land has not been planted soybean before root nodules were abundant in the uninoculated plots caused there were natural Brandyrhizobium strain in the soil.The inoculated plants have nodulation, shoot DM and grain yield values were similar when applied urea at 20 kgN/ha and higher when uninoculated

Table 12: *N-fixation capability of Rhizobium in different fertilizers application method on mungbean grown in Dong Nai and Tay Ninh provinces*

Cultivar	Nodule number / plant			Nodule mass (g/5 plants)		
	Site 2 Fe luvisol	Site 6 Ferralsol	Site 3 Acrisol	Site 2 Fe Luvisol	Site 6 Ferralsol	Site 3 Acrisol
FP + N-inoc	24	2.6[A]	13.2	0.17	n.d.	n.d.
FP-N- inoc	35	2.5	15.7	0.31	n.d.	n.d.
FP-N+049	29	2.7	14.2	0.24	n.d.	n.d.
FP-N+CB1015	34	2.5	16.7	0.28	n.d.	n.d.
OF-N+CB1015	35	2.7	15.7	0.28	n.d.	n.d.

FP- farmer practice; OF – optimum fertilizer, n.d. not done

Table 13: *The effect of rhizobial inoculation and fertilizer application on mungbean in Dong Nai and Tay Ninh provinces*

	Shoot DM (t/ha)	Grain yield (t/ha)

Cultivar	Site 2 Fe luvisol	Site 6 Ferralsol	Site 3 Acrisol	Site 2 Fe Luvisol	Site 6 Ferralsol	Site 3 Acrisol
FP + N-inoc	7.20	2.88	9.14	1.70	0.83	0.90
FP-N- inoc	6.10	2.40	7.78	1.32	0.81	0.94
FP-N+049	5.83	2.83	9.47	1.45	0.89	0.98
FP-N+CB1015	5.95	2.33	8.36	1.48	0.80	0.90
OF-N+CB1015	6.00	2.25	7.91	1.35	0.69	0.85
LSD (P = 0.05)	1.23	n.s.	1.14	n.s.	n.s.	n.s

FP- farmer practice ; OF – optimum fertilizer

The data of table 12 and 13 showed that Rhizobial inoculation had little effect on the nodulation, yield and N2-fixation of mungbean. Responses to the inoculation were small and inconsistent with strain 049 slightly better than CB1015. Uninoculated plants of mungbean were well nodulated at the three sites experiment, caused naturalised Rhizobia were in these soils, perhap resulting from long ogo cultivation of the legumes, or from natural contamination of the soil via rater and wind movement.

<u>Table 14</u>: *N-fixation capability of Rhizobium in different fertilizer application Method on soybean in Vinh Long and Dong Thap provinces*

Treatments	Nodule weight (mg/plant)			
	Vinh Long	Dong Thap 1	Dong Thap 2	Dong Thap 3
1. FP0N-inoc	0 a	29 a	0 a	5 a
2. FP+N-inoc	0 a	25 a	0 a	8 a
3. FP+CB1809	174 b	221 c	23 b	192 b
4. FP+local strain	140 b	183 bc	21 b	133 b
5. OP+CB1809	215 b	158 b	27 b	158 b
CV (%)	58	30	31	45

<u>Table 15</u>: *N-fixation capability of Rhizobium in different fertilizer application* *method on groundnut in Long An province and soybean in Vinh Long and Dong Thap provinces*

Treatments	Pod yield (t/ha)					
	Long An 1 (GN)[A]	Long An 2 (GN)	Vinh Long (SB)[B]	Dong Thap 1 (SB)	Dong Thap 2 (SB)	Dong Thap 3 (SB)
1. FP0N-inoc	3.90 a	3.13 a	3.32 a	2.37 a	0.81 a	1.34 a
2. FP+N-inoc	4.31 a	3.48 bc	4.35 b	3.12 b	1.18 b	1.73 b
3. FP+CB1809	4.55 b	3.83 cd	4.20 b	3.04 b	1.29 b	1.62 b
4. FP+local strain	5.56 b	3.83 cd	4.16 b	2.75 ab	1.26 b	1.70 b
5. OP+CB1809	5.76 b	4.23 d	4.33 b	3.07 b	1.30 b	1.70 b
CV (%)	11	3	5	9	7	8

[A]GN = groundnut [B]SB = soybean

The result from table 14 and 15 suggested there was no differences in nodulating ability between the local strain and the imported strain. Nodule mass,assessed for soybean only,reflected the difference in size of the nodules formed by the indidenous rhizobia in the uninoculated plots and those formed by the inoculant rhizobia (table 14).

The enhanced nodulation of inoculated groundnut was reflected in higher pod yields (table 15). Overall treatment 5 produced the highest yield, Treatment 3 and 4 were intermediate between treatment 5 and the two uninoculated treatment.

Table 16: *Effects of Rhizobial inoculation and fertilizer application on N-fixing and growth of groundnut in Hoa binh province*

No	Treatment	No.Nodul (Nodule/ plant)	Nodule Weight		Plant Weight	
			Fresh (g/plant)	Dry (g/plant)	Fresh (g/plant)	Dry (g/plant)
1	S1L1	72.3	0.124	0.056	40.21	10.18
2	S1L2	70	0.138	0.055	32.61	8.61

3	S2L1	55.3	0.097	0.044	35.9	10.46
4	S2L2	60.8	0.124	0.051	34.12	9.4
5	S3L1	58.1	0.115	0.047	32.7	8.7
6	S3L2	52	0.129	0.05	27.22	7.26
7	Control	49.8	0.12	0.041	25.78	6.842

Table 17: *Effects of Rhizobial inoculation and fertilizer application on growth and yield of groundnut in Hoa binh province*

No.	Treatment	Pod yield/ha (ton)	Biomass/ha (ton)
1	S1L1	3.352	5694
2	S1L2	3.4997	5013
3	S2L1	3.0447	5098
4	S2L2	2.97	4997
5	S3L1	3.052	3813
6	S3L2	3.2073	4204
7	Control	2.745	3498
LSD(5%)		0.2854	1.3994
LSD(1%)		0.396	1.9421

Note: S1: No Rhizobium+30N+10 tans FYM+90 P2O5+60 K2O+500 kg Lime

S2:Rhizobium+30N+10 tans FYM+90 P2O5+60 K2O+500 kg Lime

S3:Rhizobium+15N+10 tans FYM+90 P2O5+60 K2O+500 kg Lime

L1:Multi micro- element Fertilizer (Comix)

L2:Multi micro- element Fertilizer (Grow more)

Table 18: *Effects of Rhizobial inoculation and fertilizer application on soybean in Hoa binh province.*

Treament	yield $plot^{-1}$ (kg)			yield ha^{-1} (kg)			Biomass ha^{-1} (kg)		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
S_1L_1	1,03	1,50	0,90	1717	2500	1500	3005	2987	3388
S_1L_2	0,82	0,91	0,87	1367	1517	1450	3163	3192	3305
S_2L_1	0,65	0,92	0,65	1083	1533	1083	3157	3145	3038
S_2L_2	0,94	0,81	0,70	1567	1350	1167	3045	3220	3460

S_3L_1	0,90	1,05	0,66	1500	1750	1100	2858	2982	2940
S_3L_2	1,00	0,79	0,59	1667	1317	983	2810	2723	2987
CV %	17,1			17,1			3,9		
$LSD_{0,05}$	0,272			453,04			218,51		
$LSD_{0,01}$	0,387			644,25			310,74		

S_1 = - inoc + 30N S_1L_1= - inoc + 30N + Comix + 60P + 60K

S_2 = + inoc + 30N S_1L_2 = - inoc + 30N +886 + 60P + 60K

S_3 = + inoc + 15N S_2L_1 = + inoc + 30N + Comix + 60P x 60K

S_2L_2 = + inoc + 30N + 886 + 60P + 60K

S_3L_1 = + inoc + 15N + Comix+ 60P + 60K

S_3L_2 = + inoc + 15N + modicum 2 + 60P + 60K

The results of experiments conducted in the Hoa binh province showed Rhizobium inoculant can not only increase nodulation, nodule weight, plant weight but also increase pod yield and biomass of groundnut and soybean. In combination with an N fertiliser dose of 15 Kg/ha Rhizobium inoculant has the same yield as an N- fertiliser dose 30 Kg/ha It was found that inoculated for legumes can replace about more 15 KgN/ha of urea fertiliser.

3. Conclusions:

Vietnam cultivates about 700,000 ha of legumes annually, equally distributed between the north and the south of the country. Production is about 700,000 t (worth A$350 million). None of the crops are inoculated and all are fertilized with 30-150 kg/ha at a cost to the farmers of A$50-60 million annually. It would appear to be an unnecessay cost and one that could be substantially reduced if the practice of applying fertilizer N wase replaced by practice of inoculation. The cost of the later would be in the order of US$ 1 million annually.

For inoculation to become a reality inoculate would need to be as readity available in the market place as fertilizer N and farmer would need to be educated in their use, just as they are educated about using fertilizer and chemical. Not every legume crop would need to be inoculated. In many of the established legume areas in the country, the naturalised rhizobial already present in the soil would be adequate. In other areas, howerver, inoculation would have clear economic benefits So extension programs require information on the value, application and marketing of inoculants to farmers.