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A Time Course Study and Competition between Rhizobia-Affecting Nodulation in Two Soybean Genotypes

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A greenhouse experiment was conducted to evaluate the time course of nodulation and completion between strains USDA 123 and CB 1809 on *Williams* soybean and on P1 377578 (#671). This P1 has been shown to restrict nodulation and competition of USDA 123. The study was performed using vermiculite and fine sandy loam soil as growth media. Nodulation and competition in both medium were evaluated at 4, 6, 8 and 10 weeks. Three additional treatments included delayed inoculation of CB 1809 at 1, 2 and 4 weeks after inoculation the soil with USDA 123 were also performed. Averaged over the dates, strain USDA 123 produced 35 and 37%, respectively of the nodule mass and number on P1 377578 as compared to CB1809. In competition for nodulation in vermiculite, USDA 123 formed 43, 80, 62 and 64% of the nodules on *Williams* at 4, 6, 8 and 10 weeks, respectively, while only formed 0, 16, 9 and 16 % of the nodule on P1 # 671. In soil, USDA 123 formed 95% or more of the nodule on *Williams* through 10 weeks, while it formed 29% or less of the nodules on P1 # 671. In the delayed inoculation treatments a maximum of 14% of the nodules on *Williams* were formed by CB 1809, whereas on P1 # 671 a ratio of 89% or more were colonized by the same strain. The marked influence of the host on competition and affinity to nodulate with certain strain is demonstrated in this study.

Keywords: Soybean, Time course, Host`s mechanism, Competition.

The first step in the development of the root nodule symbiosis in the mutual recognition by the two partners a process which is rather specific. However, it is not necessary that the highly infective strain could be the highly effective one to fix copious nitrogen for plant requirements. Thus, successful nodulation and nitrogen fixation by legumes inoculated with *Rhizobium* strains depend on genetic and environmental factors. Once introduced, *Rhizobium* bacteria must multiply in the rhizosphere and infect their host plant to initiate the symbiotic process (Swelim *et al*., 1996). Success depends on competitiveness, ability to survive in the soil and compatibility of the host (Dowling and Broughton, 1986). Many effective strains have been reported to poor competitors and may consequently form very few nodules in the presence of ineffective strains

(Franco and Vincent, 1976 and Trinick, 1982). Keyser and Li (1992) mentioned that one of the major problems in nodulation technology with soybean is the establishment of an introduced inoculums strain of *B. japonicum* of the nodules of soybean grown in soil which contain indigenous population of bradyrhizobia. Thus, when the indigenous strains dominate the nodules, response to inoculation is not observed. In some instances, serogroup USDA 123 is poorly competitive in occupying nodules under certain conditions, these conditions seemed to be related to the host mechanism of nodule regulation (Olsson *et al*., 1989). Kosslak *et al.* (1983) reported that many factors of both the microsymbiote and the host play important roles in determining which strain succeeds in occupying the majority of the nodules. Furthermore, other studies have indicated that the host plant can play an important role in selecting the successful strain (Diatloff and Brockwell, 1976 and May & Bohlool, 1983). Other workers suggested that the early events in infection and nodulation are important in competition among *Bradyrhizobium japonicum* strains *(*Kossla*k et al.,* 1983 and Heron & Pueppke, 1987). In their study, Cregan and Keyser (1986) demonstrated that, variation in the time required to form nodules by particular strains of *Rhizobium* may play a key role in determining competitiveness among genotypes of soybean, they also added that indigenous strains could be restricted by the host plant and nodulation could be done by the preferable rhizobial strain maintained in the interoduced favourable inoculum. Bauer (1981) reported that the host plant respond rapidly to the challenge by *Rhizobium,* within two or four hours. It appears that the host plant can distinguish the fate of an infection at a very early stage with respect to its potential in establishing the symbiotic nitrogen-fixing state furthermore, the host plant appears to control not only the mode of infection but also the morphology and intracellular organization of the infected cell (Verma and Nadler, 1987). However, Keyser and Li (1992) recommended that the provision of superior strains for use in inoculants remains the primary applied contribution from the field of BNF. The main objectives of this study are to compare the time course of nodulation and competition among two strains of bradyrhizobia and also to evaluate the effect of delayed inoculation with an effective strain on N_2 fixation in vermiculite and sandy soil.

Material and Methods

A greenhouse pot experiment was conducted on soybean (*Glycine max* L. Merill) cultivar *Williams*-82 and the introduction P1 377578 “671” using two inocula strains USDA 123 “Pm” and a competitor one CB 1809 “E” as effective strain. The experiment was performed in sterile vermiculite (280 g pot^{-1}). A number of 64 and 92 white plastic pots 7 $^{=}$ were used for vermiculite and soil growth media, respectively. The pots were soaked overnight in 10% of Rocal as a sanitizing agent and then thoroughly rinsed with tap water. A randomized complete block design was used that consisted of a split plot arrangement of treatments. Whole plots (pots) were inoculation treatments:

- Uninoculated control “UC”.
- Strain USDA 123 premix “PM”.
- highly effective strain CB 1809 “E”.

- 123 premix + CB 1809 "Pm + E".

Subplots were soybean genotypes *Williams* and P1 377578 (#671). Each treatment was harvested at 28, 42, 56, and 70 days after planting (DAP). In soil, additional inoculation treatments were performed, 123 premix followed by delayed inoculation of CB 1809 applied either at 7, 14 and 28 DAP. Plants were harvested 6 weeks after application of the delayed inoculation. Four pots were used as replicates. Seeds were surface sterilized for 10 min. in 6% calcium hypochlorite, washed exhaustively with sterile water and pre-germinated for 48hr. on soft agar plates, 7.5 g agar liter^{-1} , and incubated at 28 ^{o}C. Two pre-germinated seeds of both soybean genotypes were sown per hole (4 holes/pot).

671

W W

671 cultivated pot

Rhizobial inoculation was done as following: a volume of 0.25 ml of the original culture USDA 123 (5.6 x10^8 cell/ml) were added to each pot after dilution with 100 ml of sterilized solution containing NaCl, $MgSO_4$ and K_2 HPO_4 (Broughton and Delworth, 1971) to obtain approximately initial count of 1 x 10^5 cell/g soil for premix treatment. The vermiculite Pm treatment treated with the same inoculum using different volume to get equal dense of rhizobial inoculums that mixed very well with soil and/or vermiculite before pot packing. For E treatment, 10 ml of CB 1809 culture added to 90 ml of yeast extract manitol broth to obtain a population of 1 x 10^8/ml, then 1 ml of the prepared inoculums was used for inoculation of each hole. Soil inoculation treatment was performed using an equal volume of culture CB 1809 for inoculation the pots pretreated with USDA 123 after 1, 2 and 4 weeks of inoculation date, then 50 ml of sterilized water were splashed on each hole containing two plants. After sowing the seeds, the pots upper surface was covered with sterilized gravels. Plants were thinned one week after to one plant per hole. Irrigation was done through the bottom of pots (into the tray at the bottom) wherever they need with N free nutrient solution through the week days. On the week end days tap water was used for watering to prevent salt accumulation (Somasegaran and Hoben, 1985). For each treatment 100 nodules were picked and analyzed for rhizobial strain occupancy by immunefluorescence "FA" with strain - specific fluorescent antibodies (Bohlool and Schmidt, 1980).

Measurements: nodule numbers and weight, top dry weight, top % N, top total N and nodule typing percentage.

Results and Discussion

Top dry weight, nodule number and nodule dry weight per plant are shown in Table 1. In respect to top weight, the control treatment UC showed that soybean genotype *Williams* and / or the introduced one “671” achieved fairly similar growth when nitrogen was limited in the soil treatment. On the other hand, inoculation with the proper rhizobial strain enhances the N_2 fixation process resulted in increasing biomass metabolism by plant. So, the interaction between strain 123 and *Williams* revealed top growth four times as much as 671 at 70 DAP. On the other hand, the interrelationship between the host 671 and the symbiont CB 1809 did much better than with strain 123 however, Williams did not, that is why strain CB 1809 was chosen as an inoculum strain because of its superior performance in a previous field trail in similar soil conducted by Cregan and Keyser (1986). The competition treatment (Pm + E) showed a similar pattern when Williams was with strain 123 alone and P1 671 with CB 1809 alone. This was also found with the delayed inoculation treatments.

TABLE 1. Top dry weight, nodule number and nodule dry weight /plant of soybean.

Treatment	Time of sampling	Top D.W. g/plant				Nodule numbers				Nodule D.W. mg/plant			
		Soil		Vermiculite		Soil		Vermiculite		Soil		Vermiculite	
		W	671	W	671	W	671	W	671	W	671	W	671
UC	4 wks	1.51	1.51	0.39	0.39	0	0	0	0	0	0	0	0
Pm" 123"		1.36	1.44	0.75	0.39	61	0	36	16	56	0	94	27
E"CB1809"		1.39	1.32	0.78	0.72	8	3	31	25	40	14	73	53
Pm + E		1.48	1.40	0.78	0.66	74	11	29	16	71	12	79	56
UC	6 wks	3.82	3.74	0.62	0.50	0	0	0	0	0	0	0	0
Pm		6.82	3.29	3.69	0.76	125	19	82	23	359	54	343	84
E		5.06	3.58	4.28	3.13	94	56	70	55	300	187	217	198
Pm + E		6.13	3.35	3.89	3.63	127	66	62	48	405	220	278	238
UC	7 wks	4.19	3.89			2	2			7	20		
Pm		9.93	4.68			211	36			729	156		
E		7.33	5.85			113	98			521	472		
Pm + E		9.41	5.13			188	95			681	436		
Pm 1 w +E		9.90	6.61			184	73			754	416		
UC	8 wks	6.01	4.82	0.66	0.55	4	6	0	1	23	55	0	2
Pm		14.65	5.82	11.02	1.63	300	34	131	22	1084	269	656	133
E		13.22	8.73	9.82	9.64	133	70	86	56	1003	645	317	173
Pm + E		13.38	6.62	12.29	7.74	206	56	68	42	830	465	469	225
Pm2w + E		12.98	8.95			268	93			1022	444		
UC	10 wks	7.93	6.51	0.80	0.64	3	3	2	1	40	51	5	3
Pm		26.04	6.43	21.62	3.88	215	27	169	10	1379	310	808	146
E		18.67	13.68	21.37	13.58	171	85	71	55	1168	804	391	296
Pm + E		25.94	16.03	21.52	12.81	207	62	97	46	1059	706	669	339
Pm4w + E		26.25	11.58			255	59			1436	305		

W: *Williams* variety. wks: Weeks . E: Reference str. CB 1809. 671: New introduction.
UC: Untreated control. Verm: Vermiculite . Pm: Pre-inoculated with str. 123.

In vermiculite medium the growth rate and consequently accumulation of plant biomass was quite different than that obtained by soil treatments for both soybean varieties at 10 weeks except in case of that treatment included P1 671 when inoculated with CB 1809, since the obtained data were much close in both soil and vermiculite. That means that the host plant P1 671 proved to be more promiscuous to rhizobial strain CB 1809 in both used media soil and vermiculite in contrast to the pattern happened with *Williams.* These data are in agreement with those obtained by Abd El-Maksoud and Keyser (2010). Generally, in contrast to soil treatment *Williams* was almost equally productive with strain CB 1809 as with strain 123. As clearly seen, P1 671 was much more productive with strain1809 than with strain 123 (13.58 and 3.88 g/plant, respectively. The reaction between the host and the symbiont still continued even in case of competition between the two strains (12.81 g/plant). In other words, the data of competition treatment look like the data of Williams inoculated with strain 123 alone and P1 671 inoculated with CB 1809 alone. In this respect, the data obtained by Kosslak *et al.* (1983) indicated that interaction which occurred during the early period of infection between soybean host and its microsymbiote are perhaps the most critical for competition among *R. japonicum* strains. The role of the host in determining the outcome of competition among strains was highlighted by the different rate of biomass accumulation for both strains interacted with each host. These results suggested that the early events in infection and nodulation are important in competition among *B. japonicum* strains.

Regards to the nodule numbers in soil treatment strain USDA 123 initiated a fast start compared to CB 1809 on Williams (61 and 8 nodules, respectively). Also, at 70 DAP the nodule number of CB 1809 on Williams still increasing compared to USDA 123. Context, perhaps CB 1809 is more sensitive to nitrate content of soil (29.06 ppm) which decreased by time through progress in plant growth. In looking at P1 671 in the competition treatment versus CB 1809 alone, the nodule numbers indicate that they probably are due to nodulation by CB 1809 rather than USDA 123. This has been previously demonstrated by Keyser and Cregan (1987) and Zodor & Pueppke (1988) however, the occupying nodule analyses by FA procedure will answer this case. Moreover, in the delayed inoculation treatments (Pm 2 w = E and/or Pm 4 w = E) the nodule numbers on 671 are more similar to their numbers with CB 1809 alone than with strain USDA 123 alone.

On the other hand, in vermiculite growth medium, the nodule numbers on soybean varieties were lower in most treatments, except those of at earlier stage of plant growth, compared to the corresponding treatments of soil. In single inoculation treatments, nodule number peak (131) was at 56 DAP (8 weeks) with *Williams* which has over 8 times as much as nodules with USDA 123 as did P1 671. As maintained in soil Williams had fewer nodules with CB 1809 than with 123. In the competition treatment most of the 671 nodules are probably from CB

1809. These findings are confirmed with obtained by Kosslak and Bohlool (1985) who established that serogroup 123 is poorly competitive in occupying nodules under certain conditions, *i.e.*, 123 to be less competitive than 110 in vermiculite or rhizobia free soil. However, Zodor and Pueppke (1988) found that nodule numbers initially increased at similar rates in soybean plants inoculated with strain 23 and 138 in sterile soil.

The nodule dry weight is closely outcomes from nodule numbers in most cases. In the uninoculated treatments some nodules occurred at 7-10 weeks. This is not expected to interfere with the obtained results in a series matter. Ingeneral, the data showed similar trends as nodule numbers. IN case of soil treatments, it was observed that strain 123 was very restricted on P1 671 compared to Williams, since the nodule weights ranged between 1/7 : 1/5 those recorded for Williams. Whereas CB 1809 nodulated well on both Williams and P1 671. In the competition treatment the nodule weight for P1 671 were fair similar to those inoculated with CB 1809 alone, a long the plant growth period. In the delayed inoculation treatments, the delay of inoculation with the effective strain CB 1809 resulted in decreasing the nodules on the root of the plant, thus the nodule dry weight of Williams was more pronounced (almost 5/1) compared to those recorded for P1 671.

Regards to vermiculite treatments, in contrast to nodule numbers, nodule biomass appeared to be steadily increasing through 70 DAP. Again, Williams formed more nodule mass with strain 123 than with CB 1809, whereas P1 did the opposite. Also, the nodule weights of competition treatment look similar to data of single inoculation of *Williams* with strain 123 and /or of P1 671 with CB 1809. Notable differences in soybean nodulation were also observed with other workers, Olsson *et. al.* (1989) who found that a 7-day delay in inoculation resulted in only 30% suppression of nodulation. In accordance with our data Kosslak and Bohlool (1984) who stated that the systemic nodule suppression seemed to be related to the host's mechanism of nodule regulation.

Table 2 shows the percentage of the soybean plants *Williams* and P1 671 in the two grown media soil and vermiculite affected with the inoculation treatment with two different strains USDA 123 and CB 1809. The amount of N fixed by legume plants depends mainly upon the successful or failure of nodulation process by the effective rhizobial strain. In case of successful nodulation by the compatible effective strain could lead to higher levels of N_2 being fixed or a greater portion of total N from BNF (Keyser and Li, 1992). The variation in N percentage may not accessible as obvious seen, it differs along the plant growth stage and the accumulative biomass. However, from the first sight it could be observed that the least values of N% were recognized in uninoculated treatment (control) due the shortage of N requirements in both growth media soil and vermiculite. It is logically that N uptake by plant continuously augments progressively along plant live. Also the N_2 fixation process was more successful in soil compared to vermiculite, since the accumulative N uptake by plant among the experiment was more pronounced in all soil treatments corresponding to those of vermiculite as clearly seen in Table 2. Among all treatments it could observed that the quantity of N uptake by *Williams* greatly surpassed that by P1 671 particularly that of the pre-inoculated treatment of soil with USDA 123 (more than 6 times). The present data were in compliance with those obtained by Abd El-Maksoud and Keyser (2010) who established that soybean genotype *Williams* is vigorous to attach with *B. japonicum* strain USDA 123, while the introduced P1 671 is restricted to that bacterium but most firmly to nodulate with other different strains (CB 1809). The increasing N uptake by P1 671 which occurred when inoculated with CB 1809 could be ascribed to the good compatibility between this strain and the host. However, the dual inoculation with the two symbionts revealed appreciable quantity of plant N uptake by the hosts compared to the single inoculation practice in both growth media soil and vermiculite. The delay inoculation treatment in soil at earlier stages of plant growth after 1 and/or 2 weeks did not exhibit any negative effect on N uptake by P1 671, but when this practice was done after 4 weeks the adverse effect on plant N uptake certainly verified, since its value sharply decreased from 359.85 to 277.12 mg/plant with decreasing rate reached to 29%. It appears that the legume plant can distinguish the fate of an infection at a very early stage of plant growth with respect to its potential in establishing the symbiotic nitrogen-fixing state as mentioned by Verma and Nadler (1987).

TABLE 2. Mean of N% &N content per plant top.

Treatment	Time of sampling	Soil				Vermiculite			
		N %		N-uptake mg/ plant		N %		N-uptake mg/ plant	
		Williams	671	Williams	671	Williams	671	Williams	671
UC	4 wks	2.40	1.68	36.16	25.28	1.65	1.17	6.35	4.51
Pm		2.75	1.88	37.10	27.03	3.63	1.65	27.37	6.31
E		2.60	1.92	35.80	25.08	3.80	4.07	29.13	29.10
Pm+E		2.74	1.87	40.22	26.06	3.68	3.58	28.55	23.24
UC	6 wks	1.21	0.91	45.80	33.75	1.07	1.00	6.51	4.93
Pm		2.52	1.03	147.37	33.67	2.70	2.31	99.16	17.31
E		2.37	2.03	119.36	73.36	2.99	2.58	124.63	80.00
Pm+E		2.77	2.15	169.74	71.69	3.11	2.46	119.86	86.35
UC	7 wks	0.78	0.73	32.56	28.23				
Pm		1.80	0.92	179.25	43.12				
E		2.56	2.35	187.74	136.75				
Pm+E		2.38	2.52	224.07	130.23				
Pm1w+E		2.39	2.31	236.04	153.60				
UC	8 wks	0.87	0.81	51.35	38.61	0.90	0.72	5.82	3.90
Pm		2.19	1.11	322.51	64.96	1.83	1.54	198.86	25.38
E		2.52	2.50	332.97	220.90	2.04	2.03	197.56	194.68
Pm+E		2.24	3.12	287.64	207.96	1.96	2.11	200.18	161.89
Pm2w+E		2.52	2.78	323.89	246.03				
UC	10 wks	0.74	0.80	58.60	52.58	0.80	0.69	6.54	4.37
Pm		2.37	1.42	602.86	91.08	1.63	1.48	351.53	55.13
E		1.89	2.69	352.13	359.85	1.77	1.81	375.26	250.13
Pm+E		2.04	1.87	524.25	298.17	1.63	2.07	351.33	264.12

Pm4w+E		1.92	2.39	497.17	277.12				

The data of typing 100 nodules/treatment of soybean plants Williams and p1 671 are presented in Table 3.In the dual inoculation treatment Pm + E the nodule typing at 4, 6 and 7 weeks in soil medium exhibited that the majority of *Williams* nodules (89 – 92 %) were occupied with strain USDA 123 whenever CB 1809 existed in few numbers of nodules (6 – 8 %). Conversely, the host P1 671 was restricted to USDA 123, since the representative nodules were the ewer compared to the compatible strain CB 1809 was actually the highest existing in its nodules (96 - 100). Similar findings were found by Zodor and Pueppke (1988) who mentioned that early nodulation kinetics of *Bradyrhizobium* strain cannot be used to predict its success in occupying nodules.

TABLE 3. Nodule typing for rhizobia strain USDA 123 and CB 189.

Treat-ment	**Time of sampling (week)**	**soil %**				**Vermiculite %**			
		Williams		**P1 671**		***Williams***		**P1 671**	
Strain		123	E	123	E	123	E	123	E
	4	92	6	0	100	43	39	0	100
Pm + E	6	92	8	2	98	80	25	16	86
	7	89	8	4	96				
Pm1wk+ E	7	98	5	4	96				
Pm+E	8	96	3	3	100	62	49	9	93
Pm2wks + E	8	95	3	2	99				
Pm+E	10	96	9	29	70	64	46	16	95
Pm4wks + E	10	89	14	13	89				

Number of 100 nodules were typed for each treatment (25 nodules/replicate).

In vermiculite medium the same trend in soil was noticed but different ratios were obtained, however the strain 123 was the preferable to *Williams* and CB 1809 to P1 671. In this respect, Verma and Nadler (1987) stated that the first step in the development of the root nodule symbiosis is the mutual recognition by the two partners – a process which is rather specific. In the delay inoculated treatment Pm 1 week + E the pre-exposure of strain 123 to soybean roots increased the nodule number occupancy of the strain to 98%, hence the nodule occupancy with E strain apparently decreased 5 %, whereas, it was the highly distinguished with P1 671. In the fourth stage after 8 weeks the dual inoculation of the symbionts did not appear any remarkable variations in both soil and vermiculite. These data are in agreement with obtained by Ham (1980) who stated that the host plays an important role in determining which strain succeeds in occupying the majority of nodules on root of legume plant. On contrast, Kosslak *et al*. (1983) reported that the pre- exposure of soybean roots to a poor competitive strain of *R. japnicum* results in a significant increase in nodule occupancy by that strain.

In the second delay inoculation treatment Pm 2 weeks + E in soil, 56 DAP, although the exposure of the host P1 671 to strain USDA 123 15 days before inclusion the strain however, it did not exceed its nodulation occupancy ratio than 2 %, whereas 99% of total nodules were infected with E strain. After 10 weeks of dual inoculation treatment in the soil and vermiculite, no obvious variation than previous tendency were obtained, except a little increase of nodule number invested with USDA 123 on roots of P1 671 (29 %) followed by parallel decrease in nodule numbers occupied by E strain (70 %). At the end of ten weeks, delay inoculation treatment Pm 4 weeks + E in soil exhibited some variations in nodule occupying ratio of both strains USDA 123 and E for both *Williams* and P1 671. The strain 123 existed in most of *Williams*'s nodules (89), whereas not much nodule numbers were occupied by E strain (14%). On the other hand, the opposite was found by the two strains on the host P1 671. In contrary to that Kosslak *et al. (*1983) established that the majority of the nodules formed on the mature plant were initiated by the strain to which the host was expressed and this occurred whether the primary inoculums was an effective or ineffective strain. These results indicate that indigenous non-effective strain could be restricted through selection of the suitable host plant and inoculation with preferable *Rhizobium* strain at the early stage of plant growth.

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دراسه على التنافس بين الميكروبات المكونة للعقد الجذريه على صنفين من فول الصويا والوقت الذى يسبق تكوين العقدة

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تم اجراء تجربة اصص لتقدير الوقت اللازم لبدء تكوين العقد الجذرية وكذلك التنافس بين سلالتين من الريزوبيا USDA123 and CB 1809 على صنفين من فول الصويا. Williams and PI#671 وقد تم إنماء النباتات فى وسطين نمو Vermiculite وتربة رملية طميية فعملية تكوين العقد وكذلك المنافسة بين الميكروب والعائل تمت دراستها بعد 4 ، 6، 8 و10 أسابيع . فى التجربة التى استعملت فيها التربة كوسط نمو تم تلقيحها بريزوبيا CB 1809 بعد تاريخ التلقيح بريزوبيا USDA 123 لمدة أسبوع ،اسبوعين وأربعة أسابيع بهدف اعطاء الفرصة للريزوبيا الملقحة اولا USDA 123 ان تقوم بعملية التعقيد للنبات ومدى مقاومة الصنف PI #671 لعملية التعقيد بهذا الميكروب حيث أن له قدرة ذاتية على مقاومة التعقيد بهذه السلالة من الريزوبيا .وقد أظهرت النتائج عند فحص العقد بواسطة فلورسنت ميكروسكوب أن وزن واعداد العقد الجذرية المتكونة على فول الصويا صنف PI#671 نتيجة التلقيح بميكروبUSDA 123 تمثل 35 ، 37 ٪ على التوالى من مجموع العقد الجذرية على الجذور ، اما باقي العقد فكانت محتويه على ريزوبيا .CB 1809 فى تجربة Vermiculite مع Williams احتلت بكتريا USDA 123 نسبة 43، 80، 62و64 ٪ من مجموع العقد المتكونة على جذور النبات وذلك بعد 4 ،6، 8 و10 أسبوع من عمر النبات ، فى حين كـــونت نفس السلالـــة عدد من العقد أقـــل فى صنف PI #671 حيث بلغت صفر ،16، 9و16 ٪ بنفس المدد المذكورة سابقا.

فى التجربة المستعمل فيها التربة كوسط نمو للنبات كونت سلالة الريزوبيا (USDA123) اكثر من 95 ٪ من العدد الكلى للعقد على جذور صنف Williams بعد 10 أسابيع ، بينما كانت العقد المتكونة على جذور صنف PI#671 لنفس السلالة تقل عن نسبة 29 ٪.

فى التجربة التى تم التلقيح فيها بميكروب CB1809 متأخرا عن التلقيح ل USDA123 بمدد مختلفة ، بلغت نسبة العقد المتكونة نتيجة التلقيح هذه 14٪ كحد اقصى على جذور صنف Williams بميكروب CB1809 فى حين بلغت هذه النسبة 89 ٪ أو اكثر على جذور صنف PI#671 وقد اظهرت هذه النتائج ان لبعض الاصناف من فول الصويا قدرة ذاتية على الحد من فعالية بعض سلالات الريزوبيا والتعقيد بها ومن ثم يمكن التغلب على بعض سلالات الريزوبيا ذات الكفاءة الأقل فى تثبيت النيتروجين الجوى والمتواجدة فى التربة الزراعية بهدف ادخال سلالات بكتيرية جديدة ذات قدرة عالية فى تثبيت النيتروجين الجوى وزيادة المحصول وتحسين انتاجيته واستعمال الاصناف النباتية التى تتواءم مع هذه الميكروبات .