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Restriction Specificity of Some Soybean Genotypes to *Bradyrhizobium japonicum* Serogrous

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***Abstract*—**Competitive relationships among *Bradyrhizobium japonicum* USDA serogroup 123, 122 and 138 were screened versus the standard commercial soybean variety *Williams* and two introductions P1 377578 "671" in a field trial. Displacement of strain 123 by an effective strain should improved N_2 fixation. Root nodules were collected and strain occupancy percentage was determined using strain specific fluorescent antibodies technique. As anticipated the strain USDA 123 dominated 92% of nodules due to the high affinity between the host and the symbiont. This dominance was consistent and not changed materially either by inoculation practice or by introducing new strainan. The interrelationship between the genotype *Williams* and serogroup 122 & 138 was found very weak although the cell density of the strain in the rhizosphere area was equal. On the other hand, the nodule occupancy of genotypes 671 and 166 with rhizobia serogroup 123 was almost diminished to zero. . The data further exhibited that the genotypes P1 671 and P1 166 have high affinity to colonize with strains 122 and 138 whereas *Williams* was highly promiscuous to strain 123.

Keywords*—B. japonicum* serogroups, Competition, Host restriction, Soybean genotype.

I. INTRODUCTION

THE legume may favor of a number of rhizobia strains to form the nodules. *Rhizobium* spp. may therefore differ in their nodulating competitiveness. The choice made by the host is not dependent on the N-fixing ability of the strain. A great number of nodules may be formed by a strain fixing little or no nitrogen even in the presence of effective strains [1]. More efficient nitrogen-fixing bacteria, *R japonicum*, seems to be one of the means of obtaining efficiency and stability of soybean yield, therefore the most commercial way to provide additional N for maximum yield is through the use of more effective strains of *R japonicum* [2] . The predominance of strains of serogroup USDA 123 in the soil growing soybean has been a critical problem [3]. Due to the variability in effectiveness that exists in the indigenous population, the main effectiveness of indigenous *Rhizobium* population varied between 62-93% of the effectiveness of a standard economical inoculants strain [4]. It is of a great importance to attempt to improve the effectiveness of the symbiosis by introducing more effective strains, in order to displace those less effective population of *Rhizobium* already established in more soybean growing soils. The relative success of different strains of *R. japonicum* is probably the result of many factors interacting to affect rhizobial survival and root colonization as well as competition for nodule occupation.

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Such factors are obviously of great importance and their role must be defined and clarified. Competition between effective strains of root nodule bacteria to nodulate the host plant is a complex phenomenon which occurs between strains of the natural soil population and inoculum strains. Since some decades, reference [5] mentioned that the most difficult situation in the use of rhizobial inocula occurs when it becomes necessary to establish and maintain strain in a soil containing the same rhizobial species. The establishment is particularly difficult if the natural population is already large at planting time. Ten years after**,** reference [6] concluded that, inoculation of soybean with selected strain of *R. japonicum* has been notably unsuccessful in influencing nodulation or enhancing N_2 fixation in regions where soybean have been cultivated previously. The inoculants fails to nodulate the soybean to any significant existent, where as virtually all of the nodules are occupied by strains indigenous to the soil. It was also mentioned that *B. japonicum* serotype USDA 123 is successful as an indigenous soil organism and is highly successful in outcompeting other soybean symbiont strains introduced as rhizobial inocuants. Reference [7] stated that, throughout much of the major soybean production area of the US, indigenous soil strains of *R. japonicum* serogroup 123 dominate nodulation of soybean regardless of the inoculation practice or of the cultivar grown. Although, that situation of the serotype strain USDA 123, reference [8] found that, averaged across three soybean cultivar growing in the soil free of indigenous soybean rhizobia, seed yield with USDA 123 ranked last among seven strain tested. A reduction in nodulation by this highly competitive serogroup would allow nodulation by other strains and might provide a mean of significantly increasing the proportion of nodules containing the inoculum strain [9]. Reference [10] found that nodules occupancy and nodulation characteristics were influenced by plant genotype, bacteria strain and environment. In a greenhouse experiment containing soil and seed applied application, lateral - root nodulation of the restriction host P1 1371607 by USDA 123 was not significant [11]. Reference [12] stated that host control of restricted nodulation and reduced competitveness is quite specific and effectively discriminates between *B. japonicum* strains which serologically related.

II. MATERIAS AND METHODS

In a field experiment, three genotypes of soybean *(Glycine max.),* included the standard commercial soybean variety *Williams* and two introductions P1 377578 "671" and P1 371607 "166" were screened for their ability to nodulate with

Bradyrihizobium japonicum mixed strains USDA 123, 122 and 138 through the competition between these different strains. The three rhizobial strains were examined for purity by inspection of single colony isolates on yeast manitol agar medium, YMA, and were verified by reaction with specific fluorescent antibodies technique FAs [13]. Soybean cultivar Williams and P1 671 were grown for two successive seasons in fine sandy loam soil, where the third cultivar P1 166 introduced in the second season. The soil had no detectable *B. japonicm* (<10 cell g/ dry soil) as determined by MPN using the plant infection test [14]. Seeds were surface sterilized for ten min. in 6% calcium hypochlorite solution and washed five times with sterilized distilled water. The soil fertilized with the recommended dose of K & P. Four treatments were suggested; each included four replicates in a randomized complete block experimental design. The treatments consist of uninoculated control – UC, premix treatment inoculated with *Rhizobium* strain USDA 123 – Pm (the predominant ineffective strain in the soybean fields), granular peat inoculated treatment containing the two mixed cultures of the highly effective strains USDA 122 and 138 – G and the fourth one included Pm + G. The Pm plots had been inoculated with liquid culture of USDA 123 before planting to develop in a high population in the seed bed in an attempt to create a situation similar to that soil of ever grown soybean. The granular peat inoculum that contains the two strains of USDA 122 + 138 was applied to the seeds at planting. Rhizobial inoculation practice in the fourth treatment was done in successively as followed in the treatments 2 and 3. At complete flowering, plant samples were removed from the replicates of the representative treatments plots. The roots were gently clarified out of soil particles and then washed carefully. Pecking up 50 nodules for each replicate was done for examination by FAs technique for detection of strains occupation percentage of the three soybean genotypes.

The objective of this work was to benefit of the host restriction specificity of some soybean genotypes to overcome the highly competitiveness potency of an ineffective predominant serogroup 123.

III. RESULTS

Table 1 shows the nodule occupancy percentage of *Bradyrhizobium japonicum* serogroups USDA 123, 122 and138 which nodulated soybean genotype Williams and plant introduction P1 671 in the first growing season. As clearly seen from the Table that very few nodules were formed on the roots of uninoculated plants both W*illiams* and/or P1 671, particularly those contain strain 123 and/or 138. However, there were relatively more nodule numbers occupied by strain 122.

TABLE I
NODULE OCCUPANCY*PERCENTAGE *B .japonicum* SEROGROUPS in SOYBEAN GENOTYPES
1st Season

Treatments	Soybean genotypes					
	Williams			PI 377578"671"		
	nodule occupation *% of R.* serogroups					
	123	122	138	123	122	138
Unionculated "UC"	3	15	9	0	31	2
Premixed with USDA 123"Pm"	92	2	0	12	38	18
Granullar peat with 122 &138"G"	3	49	23	0	56	14
Pm+G	76	12	6	1	66	23

*Nodule occupancy% average of 50 nodules x 4 replicates.

In case of premixed inoculation treatment with str. 123, it was found that almost the whole number of nodules on *Williams*,s root occupied with 123, since the nodule number percentage reached to 92%, where the same strain (123) occupied only 12% of the total nodule in p1 671. In contrary to that were the strains 122 and 138, since scarce nodules contained strain 122 (2), but nothing nodules were occupied with 138 (0). However, strain no. 122 was the preferable symbiont to P1 671 (38%) followed by 138 (18%)

Whereas the inoculum consists of granular peat with the mixed cultures of strains 122 and 138 exhibited different criteria. In absence of str. 123, Williams moderately nodulated with str. 122 followed by 138 (49 and 23%, respectively. The same trend was observed with P1 671 but nothing nodule was occupied with 123.

On the other hand the Pm + G mixed treatment, the genotype Williams exhibited a large affinity to nodulate with USDA serogroup 123 (76%) and with moderate percentage (12%) of serogroup 122 and as little percentage with str. 138. Whereas, in an opposite situation, the genotype P 671 had a different occupation percentages, being 1, 66 and 23 for serogroups 123, 122 and 138, respectively.

The data of the second season included new introduced genotype P1 371607 "166" as shown in Table 2. The pattern of host rhizosphere colonization by the three population of competing *R. japonicum* was similar for both growing seasons. All soybean genotypes exhibited no nodules of strain 123 on their roots in case of uninoculated treatment "Control". Very few nodules of strain No. 122 and 138 were predicted on the plant roots of genotype P1 671 and 166. In case of Pm treatment the strain No. 123 was the most dominant symbiont with three genotypes. The occupation percentage of strain 123 reached their maximum (100%) in case of Williams cultivar decreased to 83 and 76% with genotype P1 671 and 166, respectively. Whereas, scant percentage of *B. japonicum* serogroup 122 and 138 were detected with the two introduced soybean hosts.

TABLE II
NODULE OCCUPANCY*PERCENTAGE *B .japonicum* SEROGROUPS in SOYBEAN GENOTYPES
2nd Season

Treatments	Soybean genotypes								
	Williams			PI 377578"671"			PI 371607"166"		
	B. japonicum USDA%								
	123	122	138	123	122	138	123	122	138
Uninoculated "UC"	0	0	0	0	3	3	0	6	5
Premixed with USDA 123"Pm"	100	0	0	83	1	2	76	6	8
Granullar peat with 122 &138"G"	0	56	44	0	57	45	0	58	40
Pm+G	76	20	11	9	59	48	5	72	35

*****The percentage exceeded than 100% could be due to the double occupancy.

In contrary to that, the serogroup 123 disappeared in all soybean genotype within the cultivated plots treated with the granular peat contained rhizobia serogroup 122 and 138 . More than 50% of examined nodules of the three soybean genotypes were occupied with serogroup 122 with no remarkable differences. The serogroup 138 occupied the second rank, since the occupation percentage ranged between 40 – 45% through the three soybean genotypes. In case of premixing the soil with the strain 123 in addition to the granular peat contains strains 122 + 138, this treatment reduced the occupation percentage of *Williams* with strain 123 from 100 to 76%. These decreases were more pronounced with the other two genotypes P1 671 and 166, since it was promptly decreased from 83 – 9% and from 76 – 5%, respectively. In *Williams*, the strain 122 cited after 123 followed by that one of 138. Whereas, prevalence was to the serogroup 122 followed by str. 138 in both soybean genotypes P1 671 and 166. The data proved that P1 was more promiscuous to str. 122 than P1 671. In opposite trend, was the strain 138 since it was occupied a percentage of 48 with P1 671 which was larger than that detected with P1 166 (35%).

IV. DISCUSSION

Unless a way is found to enhance competition of introduced strains with those ineffective strains dominant in the soil variety competition are anemic and the selection of better soybean variety could be hampered because their performance could be affected by soil rhizobia of unknown effectiveness. One can only speculate about how many genotypes of soybean have been chosen on the basis of their efficient symbiosis with a naturalized population at a particular location, and then have failed to meet expectations when tested in the other areas where the rhizobial population is different. It is noteworthy that, the choice made by the plant is not dependent on the nitrogen ability of the strain [1]. A great number of nodules may be formed by a strain fixing little or no nitrogen, even in the presence of effective strains. It was found also that the relative success of different strains of *R. japonicum* is probably a result of many factors interacting to affect rhizobial survival and root colonization as well as competition for nodule sites. Such factors are obviously of great importance and their role must be defined and clarified.

The present data showed significant differences among plant genotypes in their acceptance of *R. japonicum* strains of specific serogroups. Closely related soybean genotypes P1 671 and 166 had similar distribution of *R. japonicum* in their nodules. The data also indicated specific interactions between soybean genotypes and *R. japonicum* serogroups in nodule formation. The same findings were found by reference [15] who indicated that soybean genotype may be used to exclude specific isolates or subpopulation of serocluster 123. As mentioned by references [16], [17] at nodule formation, the legume may favor one of number of strains of *Rhizobium* to form the nodules. *Rhizobium* spp. may therefore differ in their capacity to be selected by the plant host i. e. in their nodulating competitiveness.

The data further proved that Williams genotype soybean is vigorous to attached with *B. japonicum* strain USDA 123, while the introduced genotype soybean P1 671 are restricted to that bacteria, while they are most firmly to nodulate with the more effective rhizobial strains USDA 122 & 138. Early, reference [18] showed differences among plant genotypes in their acceptance of *R. japonicum* strains from a mixed naturalized population in the soil. Specific interactions were also noted between soybean genotype and *R. japonicum* serogroups in nodule formation. Closely related soybean genotypes had similar distribution of rhizobia in their nodules. The present data are confirmed to that obtained by reference [9] who postulated that it is possible to restrict nodulation by the predominant str. USDA 123 at growing soybean genotypes through performing a specific inoculums contains the more effective rhizobial strains. Reference [15] added that host control of restricted nodulation and competitiveness reduction is quite specific and effectively discriminates between *B. japonicum* strains which are serologically related.

In conclusion, a soybean line that is compatible with the strains in the soils where it is being tested could perform better, and have a greater chance of being selected, than a line whose genotype is not as compatible with the strain in the testing soil. It is also be necessary to mention that, this compatibility should considered when introducing *R. japonicum* strains into new areas.

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