QUANTITATIVE ASSESSMENT OF BIOLOGICALLY FIXED NITROGEN BY SOME BACTERIA STRAINS OF *RHIZOBIUM* FOR BEANS, USING THE ISOTOPIC METHOD

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ABSTRACT

The nitrogen fixing capacity and efficiency of seven bacteria strains inoculating the beans to fix the nitrogen from air, were tested in a field experiment at the Agricultural Research Station in the Teleorman county. To determine the quantity of the biologically fixed nitrogen (q b f N), the technique of the isotopic dilutions was applied using the nitrogen stable isotope (^{15}N). According to our determinations, the nitrogen quantity biologically fixed in the total biomass of plant depends on the bacteria strain, from 63.2 kg/ha (FL 8 strain) to 77.3 kg/ha ($CIAT_{899}$ strain). The index of the simbiotically fixed nitrogen yield (% q b f N) reached the highest values (80.0 - 82.1%) with the FL_{23} and FL_{400} strains, treatments that also secured the highest yield level.

Key words: bacteria strain, biologically fixed nitrogen, nitrogen fixing capacity, isotopic method.

INTRODUCTION

This study was carried out within the research programme called "Increase of soil fertility and yields by higher management of nitrogen fixing bacteria of *Rhizobium* genus ", coordinated by IAEA - Vienna and aiming at the increasing the contribution of the biologically fixed nitrogen to form the beans yield. The action ways in this view have been oriented to selecting new bacteria strains with a good and sustainable efficiency in nitrogen fixing under field conditions, as well as to improve the nitrogen nutrition of the beans by using the two complementary ways - the nutrition with biological nitrogen and the assimilation of the combined nitrogen in soil (Popescu, 1996).

The research carried out at the Agricultural Research Station of Teleorman in 1996-1997, an integrated part of the above mentioned programme, had in view to assess the capacity to fix the nitrogen from air and the field performances of some *Rhizobium simbionte* strains for beans, taking into account the fact that the Romanian soils are populated with numerous saprophyte populations of *Rhizobium simbionte*, which represent active competitors for the bacteria strains selected and used as inoculants (Popescu, 1997).

MATERIALS AND METHODS

The research was carried out in the experimental field of the Teleorman Agricultural Research Station on cambic chernozem with a high natural fertility (232 ppm K, 31 ppm P, NI = 3.6) and a neutral soil reaction. The climate is droughty and warm with the mean annual temperature between 10.5^0C and 11.0^0C, and mean annual precipitation between 500 and 550 mm.

To assess the nitrogen fixing capacity of the seven tested bacteria strains, the procedure of the isotopic dilutions was used having as a base the stable isotope of the 15 mass nitrogen (^{15}N), (Hera, 1980; Hera et al., 1981).

The experiment was organized according to the method of randomized blocks with three replicates. The size of each plot was 15 m^2 of which 2 m^2 received fertilizers with marked nitrogen (^{15}N). The control plant, a non fixing nitrogen one, was the oats for grains. The used source of ^{15}N was the ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) with a concentration from 10.132 per cent ^{15}N atoms in excess, applied to the beans at a rate of 20 kg/ha with 5.066 ^{15}N atoms in excess, and to the oats at a rate of 100 kg/ha with 10.132 per cent ^{15}N atoms in excess. In autumn, 80 kg P_2O_5/ha were applied by ploughing.

The seven tested bacteria strains taxonomically belong to three species of the *Rhizobium* genus, namely:

- FL_8; FL_{23}; RCR_{3644}; $CIAT_{75}$ and $CIAT_{161}$ from *Rhizobium leguminosarum* bv.*phaseoli* ;
- $CIAT_{899}$ from *Rh. tropici* ;
- FL_{400} from *Rh.* species.

Four of the seven strains (RCR_{3644} ; $CIAT_{75}$; $CIAT_{161}$ and $CIAT_{899}$) proceed from

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the Seibersdorf Laboratory of IAEA, and the rest (FL_8 ; FL_{23} and FL_{400}) were isolated from the saprophytic rhizobial populations present in the Romanian soils by the special laboratory of the Research Institute for Cereals and Industrial Crops of Fundulea, which also mediated the execution of the chemical analyses in the IAEA Laboratory - Vienna, regarding the content of ^{15}N atoms in excess and total nitrogen in the plant samples.

The inocula for each bacteria strain were prepared with a number of 1 x 10^9 bacteria/ml and applied in suspension on seeds just before sowing. The used beans cultivar was the Romanian cultivar - Aversa, sown with a density of 50 germinable grains per 1 m^2 on April 15-18. The crop management included two mechanical weedings and the application of the Pivot herbicide at a rate of 0.5 l/ha.

At the three stages of plant growing (beginning of flowering, complete flowering and physiological maturity) the plant samples were collected (10 plants/replication) in order to carry out observations and biometrical determinations on the installation and evolution of the symbiosis, the modifications of some morpho-physiological indices and the total and useful biomass accumulated in plant. Thus, the symbiosis evolution was studied by determinations regarding the total number of nodules and the number of active nodules, and the dry weight of the fixing tissue in the first two growing stages, when also the size of the foliar area (cm^2/plant) was determined, as well as the dry biomass quantity accumulated in the whole dry plant and in the component parts of the plant.

At the maturity stage, on the basis of the chemical analyses regarding the total nitrogen content in seeds and secondary part of the plant and the content of the ^{15}N atoms in excess, it was possible to calculate, on the basis of the formula indicated by the special literature, the quantity of the total nitrogen and of the nitrogen derived from fertilization (N d f f), as well as the total nitrogen yield index and the coefficient of the nitrogen utilization from fertilization. By using the formula of Fried and Broechart (1975), improved by Hardarson and Danson (1993), the biologically fixed nitrogen quantity in plant (nitrogen derived from atmosphere- N d f a), thus assessing the efficiency of the tested strains on the basis of the index of nitrogen yield derived from the atmosphere (I N i d f a).

The significance assessment of the differences between the variances was made by the variance analysis method and the Duncan's multiple range test.

The index of the biologically fixed nitrogen yield was calculated as a ratio between the quantity of nitrogen derived from the atmosphere contained in the useful yield and the quantity of nitrogen derived from the atmosphere contained in the total biomass (Popescu et al., 1990).

RESULTS AND DISCUSSIONS

The positive results obtained within the activity aiming to scientifically assess the response of the beans for grains to seed bacterization with selected bacteria strains led to the conclusion that also, in the presence of saprophytic rhizobial populations in soil, the practice of seed bacterization can be recommended as being beneficial to yield formation and its sustainability.

The research carried out at the Teleorman Agricultural Research Station included also observations regarding the symbiotic evolution in the variants inoculated with the selected strains belonging to the above mentioned three species as well as the quantitative determinations of the total nitrogen content and of the biologically fixed nitrogen in the total and useful biomass accumulated in the beans plants.

According to the obtained data, the first occured nodules were observed on plants of the variants inoculated with *Rh. tropici* $CIAT_{899}$, 13 days after plant emergence (May 11). For the rest of the inoculated variants, the nodule occurrence was 4-8 days later.

The determinations carried out at the begining of flowering (June 8) on the total nodule number, active nodule number on plant, as well as on the dry weight of fixing tissue, expressed in mg/plant (Table 1) show nodule formation both in the inoculated variants and in the non-inoculated controls where the infection was produced by the bacteria in the soil native flora.

According to the statistical calculation, the inoculation with the experimented bacteria strains significantly influenced the values of the three analysed parameters. The highest total

Table 1. Number of nodules on plant and their dry weight at beans (Aversa cultivar) inoculated with different bacteria strains. Stage : beginning of flowering

Treatment	Total nodules no/plant	Native nodules no/plant	Active nodules %	Dry weight of nodules, mg/plant
Non-inoc. x non-fert.	6.00 d	5.00 d	70	6.60 de
Non-inoc. x fert.	6.33 d	6.33 cd	100	8.00 cd
FL_8	8.67 cd	8.67 cd	100	4.43 e
FL_{23}	17.67 b	17.67 b	100	11.87 b
FL_{400}	6.67 d	6.67 cd	100	24.33 a
FL_{3644}	8.67 cd	8.67 cd	100	6.80 de
$CIAT_{75}$	9.00 cd	9.00 cd	100	6.70 de
$CIAT_{161}$	11.00 c	10.67 c	97	5.60 de
$CIAT_{899}$	22.67 a	22.67 a	100	9.93 bc
F Factor	14.467**	16.216**		14.892**
LSD 5%	4.13	4.06		2.78
LSD 1%	5.68	5.60		3.83
LSD 0.1%	7.82	7.71		5.27

Table 2. Foliar area and accumulated biomass at beans (Aversa cultivar) inoculated with different bacteria strains Stage : beginning of flowering

Treatment	Foliar area	Accumulated biomass (mg d.m./pl.)			
	cm^2/pl.	roots	stem	leaves	total
Non-inoc. x non-fert.	171.0 d	187.3 bcd	440.7 b	827.3 e	1455.3 d
Non-inoc. x fert.	230.0 b	159.3 b	519.7 b	946.0 d	1625.0 c
FL_8	270.0 a	169.3 cd	699.3 a	1109.3 c	1977.7 b
FL_{23}	182.7 cd	173.3 cd	640.3 a	794.3 e	1612.0 c
FL_{400}	271.3 a	202.0 bc	721.7 a	1309.0 b	2232.7 a
FL_{3644}	224.7 b	249.7 a	651.3 a	1047.0 c	1948.0 b
$CIAT_{75}$	230.3 b	176.0 cd	534.3 b	929.3 d	1639.7 c
$CIAT_{161}$	240.0 c	194.7 bcd	519.3 b	833.3 e	1547.3 a
$CIAT_{899}$	290.3 a	224.0 ab	724.3 a	1388.7 a	2337.0 a
F value	24.176**	4.963**	10.866**	86.88**	67.69**
LSD 5%	25.04	38.40	94.68	68.67	115.23
LSD 1%	34.49	52.90	130.42	94.59	158.72
LSD 0.1%	47.48	72.82	179.54	130.23	218.51

nodule number as well as the active nodule number on plant were recorded in the variant inoculated with the $CIAT_{899}$ strain, followed by the variant inoculated with the FL_{23} strain. As concerns the dry weight of the fixing tissue, the highest values were recorded in the variant inoculated with the FL_{400} strain followed by the FL_{23} and $CIAT_{899}$. The results of the determinations on the plant growing and developing, in this study, are presented in table 2.

The foliar area and the accumulated biomass in roots, stems and leaves as well as in the whole plant present significant differences between the inoculation treatments. The highest values of the analysed parameters were recorded in the case of inoculation with the $CIAT_{899}$ strain, the other strains having also positive influences on some of these parameters.

In the next analysed stage - complete plant flowering - the total number of nodules, the number of active nodules and the dry weight of the nodules on plant (Table 3) significantly varied under the inoculation influence, the differences between the treatments exceeding the threshold of the significance P=5%. The highest values for all the parameters were recorded in the case of inoculation with the $CIAT_{899}$. Foliar area developed on plant in this stage as well as the biomass accumulated in the plant component parts (roots, stems, leaves and in the whole plant) had the highest values with the treatment inoculated with the FL_8 (Table 4). Also the control (fertilized and non-inoculated) had the same high values of the total biomass accumulated in plant.

At the maturity stage, the results of the statistical calculation applied to the whole accumulated biomass (Table 5) do not show significant differences between the inoculated treatments. Higher values were recorded in the non-

inoculated x fertilized and inoculated with the FL_{23} strain, the differences in these two

Table 3. Number of nodules on plant and their dry weight at beans (Aversa cultivar) inoculated with different bacteria strains

Stage : complete flowering

Treatment	Total nodules no/pl.	Active nodules no/pl.	Active nodules %	Dry weight of nodules mg/pl.
Non-inoc. x non-fert.	21.0 e	17.1 c	81	26.7 f
Non-inoc. x fert.	25.0 d	17.4 c	70	27.0 f
FL_8	40.7 bc	30.3 bc	74	40.7 bcd
FL_{23}	30.3 cd	17.0 c	56	29.0 ef
FL_{400}	26.7 d	22.0 c	82	33.0 def
FL_{3644}	43.0 b	27.7 c	64	48.0 b
$CIAT_{75}$	34.0 bcd	22.0 c	65	27.6 f
$CIAT_{161}$	41.0 bc	28.3 bc	69	39.0 be
$CIAT_{899}$	105.7 a	74.3 a	70	60.0 a
F factor	38.615**	15.117**		8.536**
LSD 5%	11.71	13.50		10.44
LSD 1%	16.14	18.59		14.37
LSD 0.1%	22.21	25.60		19.79

Table 4. Foliar area and accumulated biomass at beans (Aversa cultivar) inoculated with different bacteria strains

Stage : flowering

Treatment	Foliar area cm^2/ pl.	Accumulated biomass (mg d.m. / pl.)			
		roots	stem	leaves	total
Non-inoc. x non-fert.	468.0 c	0.349 bcc	2.53 cd	2.07 de	4.949 cd
Non-inoc. x fert.	450.7 c	0.356 bc	2.24 d	5.19 a	7.780 a
FL_8	804.0 a	0.524 a	4.44 a	3.57 b	8.534 a
FL_{23}	469.7 c	0.301 c	2.19 d	1.94 de	4.437 d
FL_{400}	607.7 b	0.374 bc	3.10 bc	2.40 cd	5.868 bc
FL_{3644}	410.7 c	0.427 b	2.76 cd	1.86 e	5.047 cd
$CIAT_{75}$	552.7 b	0.373 bc	3.43 b	2.75 c	6.556 b
$CIAT_{161}$	440.3 c	0.326 c	2.62 cd	1.83 e	4.779 d
$CIAT_{899}$	554.0 b	0.333 c	2.71 cd	2.30 cde	5.349 cd
F Factor	25.867**	6.226**	12.327**	37.942**	15.772**
LSD 5%	71.66	0.08	0.59	0.53	1.07
LSD 1%	98.71	0.11	0.82	0.73	1.48
LSD 0.1%	135.89	0.15	1.13	1.01	2.04

Table 5. Biomass production, nitrogen per cent and total nitrogen quantity at beans (Aversa cultivar) inoculated with different bacteria strains of *Rhizobium* genus.

Stage : maturity

Treatment	Dry biomass, kg/ha			Yield index %	Nitrogen %		Total nitrogen kg/ha			Nitrogen yield index, %
	seeds	second prod.	total plant		seeds	second prod.	seeds	second prod.	total	
Non-inoc. x non-fert.	1755	2295	4050	43.8	3.52	1.18	61.77	27.12	88.89	69.5
Non-inoc. x fert.	2040	2710	4750*	42.9	3.56	1.34	72.62	36.33*	108.95*	66.7
FL_8	2295**	2155	4450	51.6	3.42	1.23	78.49*	26.67	105.16*	74.6
FL_{23}	2348**	2437	4785*	49.1	3.50	1.14	82.18**	27.89	110.07**	74.7
FL_{400}	2249*	1931	4180	53.8	3.36	1.33	75.57*	25.80	101.37	74.5
FL_{3644}	2205*	2030	4235	52.1	3.60	1.10	79.38**	22.25	101.63	78.1
$CIAT_{75}$	2266*	1969	4239	53.5	3.60	1.34	81.57**	26.35	107.92*	75.6
$CIAT_{161}$	2040	2085	4125	49.5	3.67	1.49	74.85*	31.10	105.95*	70.6
$CIAT_{899}$	2134	2486	4620	46.2	3.63	1.43	77.46*	35.58*	113.04**	68.5
LSD 5%	388	422	701				12.4	8.30	15.7	
LSD 1%	534	717	823				17.0	12.51	21.6	
LSD 0.1%	744	999	1154				23.8	18.80	30.2	

cases as compared with non-inoculated x non-fertilized control exceeding the threshold of the 5% significance. The data analysis regarding the useful yield reveals significant differences as compared to the non-inoculated x non-fertilized control in the case of the treatments inoculated with FL_8, FL_{23}, FL_{400}, FL_{3644}, $CIAT_{75}$ and the lack of significance with the non-inoculated x fertilized treatment. This fact shows that the biologically fixed nitrogen preponderantly accumulates in useful yield. The higher values of the yield index denote by themselves a higher performance of the tested strains in nitrogen supplying the plants, being obviously remarked FL_{400}; $CIAT_{73}$; FL_{3644} and FL_8. The lowest yield index was recorded with the fertilized and non-inoculated treatment.

The total nitrogen content in the secondary production and the useful yield (kg/ha) are presented in the same table. The data analysis reveals significant differences of the total nitrogen amount contained in the secondary yield as compared to the non-inoculated and non-fertilized only in the case of non-inoculated x fertilized treatment and the treatment inoculated with $CIAT_{899}$. In the case of useful yield, the total nitrogen quantity in the inoculated treatments reveals significant differences as compared to the non-inoculated x non-fertilized control. The nitrogen yield index considered the most synthetic to assess the symbiosis efficiency, calculated as the ratio between the nitrogen content of the useful yield and the nitrogen quantity in the total biomass, presents higher values in the inoculated treatments with a maximum of 78.1% in case of the FL_{3644} treatment as compared with 66.7% in the case of non-inoculated x fertilized treatment. The lowest index of the nitrogen yield, similar with that of the non-inoculated, was recorded in case of the $CIAT_{899}$ strain, while this strain presents the highest values of all the indices analysed in the phases of early flowering and complete flowering, previously presented.

The percentage of the nitrogen derived from the fertilization (% N d f f), calculated and presented in table 6 indicates the highest values in the fertilized x non-inoculated fertilized, and the quantity of nitrogen derived from the fertilizers into the total biomass recorded negatively significant in most inoculated treatments, while they received the same rate of 20 kg a.i. N/ha when seeded. Therefore, the efficient use coefficient of nitrogen in fertilizers presents lower values in the inoculated treatments as compared to the non-inoculated x fertilized control.

The calculation of the symbiotically fixed nitrogen quantity on the basis of the Hardarson and Danso (1993) (Table 7) varies between 61.0 kg/ha and 77.3 kg/ha. The biologically fixed nitrogen increases in the total biomass in the inoculated treatments as compared to the non-inoculated control are between 4 per cent (FL_8 strain) and 27 per cent ($CIAT_{899}$ strain). According to the statistical calculation, the biologically fixed nitrogen quantities present positively significant differences as compared to the non-inoculated control (which establishes a symbiosis with the bacteria in the soil native flora), in case of five of the seven tested strains, excepting the FL_8 and $CIAT_{161}$ strains. The bio-

Table 6. Accumulation of ^{15}N in plants, percentage and quantities of nitrogen derived from fertilization (Ndff) and utilization coefficient of nitrogen from fertilizers at beans (Aversa cultivars) inoculated with different bacteria strains

Treatment	Excess of ^{15}N (%)		N d f f (%)		N d f f (kg/ha)			Utilization coeff.of nitrogen from fertilizer (%)	
	seeds	second prod.	seeds	total plant	seeds	second prod.	total plant	seeds	second prod.
Non-inoc. x non-fert.	-	-	-	-	-	-	-	-	-
Non-inoc. x fert.	0.390	0.342	8.42	7.39	6.11	2.68	8.79	30.6	13.2
FL_8	0.293	0.288	6.32	6.22	4.96	1.65	6.61	24.8	8.3
FL_{23}	0.229	0.236	4.94	5.10	4.05	1.42	5.47^{0}	20.2	7.1
FL_{400}	0.221	0.219	4.77	4.73	3.60	1.22	4.82^{00}	18.0	6.1
FL_{3644}	0.249	0.229	5.37	4.94	4.26	1.10	5.36^{0}	21.3	5.5
$CIAT_{75}$	0.233	0.238	5.03	5.14	4.10	1.35	5.45^{0}	20.5	6.8
$CIAT_{161}$	0.298	0.250	6.44	5.40	4.82	1.68	6.50	24.1	8.4
$CIAT_{899}$	0.205	0.216	4.42	4.66	3.42	1.66	5.08^{00}	17.1	8.3
LSD 5%							1.15		
LSD 1%							1.49		
LSD 0.1%							2.13		

Table 7. Quantity of symbiotically fixed nitrogen in different inoculation treatments according to formula of Hardarson and Danso (1993)

Treatment	Biologically fixed N_2						Index of fixed N_2 yield (%)
	seeds		second. prod.		total biomass		
	kg/ha	% of control	kg/ha	% of control	kg/ha	% of control	
Non-inoc. x non-fert.	-	-	-	-	-	-	-
Non-inoc. x fert. (control)	36.5	100	24.5	100	61.0	100	59.8
FL_8	49.1	135	14.1	56	63.2	104	77.7
FL_{23}	58.8	161*	12.8	52	71.6	117*	82.1
FL_{400}	56.4	155*	14.1	58	70.5	116*	80.0
RCR_{3644}	55.0	151*	16.9	69	71.9	118*	76.5
$CIAT_{75}$	54.7	150*	18.1	74	72.8	119*	75.1
$CIAT_{161}$	47.1	129	20.9	85	68.0	111	69.3
$CIAT_{899}$	57.9	159*	19.4	79	77.3	127*	74.9
LSD 5%	13.7	37.5	9.4	38.4	9.5	16.1	
LSD 1%	22.8	62.5	12.8	52.2	22.0	36.0	
LSD 0.1%	35.6	97.5	17.9	73.0	31.1	50.9	

logically fixed nitrogen increase in the useful yield was recorded in the inoculated treatments between 29 per cent and 61 per cent, the highest increase being recorded in case of FL_{23}, followed by $CIAT_{899}$ and FL_{400}.

As concerns the secondary production, the biologically fixed nitrogen quantity decreases in the treatments inoculated with selected strains from 52 to 85 per cent from the control value, confirming again the contribution of the biologically fixed nitrogen to the formation of useful yield.

The index of the biologically fixed nitrogen yield reached the maximum 82.1 per cent with the treatment inoculated with the FL_{23} strain, followed by the FL_{400} strain with 80.0 per cent. It should be mentioned that the $CIAT_{899}$ strain which recorded the highest quantity of nitrogen fixed in the total biomass - 77.3 kg/ha, does not prove to have an increased efficiency in field, its index of the nitrogen fixed in the useful yield reaching only 74.9 per cent being ranked the last but one place within the seven tested strains.

CONCLUSIONS

The quantity of biologically fixed nitrogen to beans in the treatments inoculated with series of the tested bacteria strains varied between 63.2 kg/ha (FL_8) and 77.3 kg/ha ($CIAT_{899}$).

The best efficiency in fixing the nitrogen from air, seen from the viewpoint of the index of the fixed nitrogen yield, was observed in case of the FL_{23} strain where I N i d f a = 82.1, followed by the FL_{400} strain.

The low value of the index of nitrogen yield derived from the atmosphere of only 59.8 per cent in the control confirms the scientific data obtained for a long time in assessing the beans response to seed inoculation with selected bacteria strains showing also that, in the presence in soil of the natural rhizobial populations, this practice is recommended, being beneficial to the yield formation and sustainability.

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Table 1. Number of nodules on plant and their dry weight with beans (Aversa cultivar) inoculated with different bacteria strains Stage : beginning of flowering

Treatment	Total nodules nr./plant	Native nodules nr./plant	Active nodules %	Dry weight of nodules mg./plant
Non-inoc. x non-fert.	6.00 d	5.00 d	70	6.60 de
Non-inoc. x fert.	6.33 d	6.33 cd	100	8.00 cd
FL_8	8.67 cd	8.67 cd	100	4.43 e
FL_{23}	17.67 b	17.67 b	100	11.87 b
FL_{400}	6.67 d	6.67 cd	100	24.33 a
FL_{3644}	8.67 cd	8.67 cd	100	6.80 de
$CIAT_{75}$	9.00 cd	9.00 cd	100	6.70 de
$CIAT_{161}$	11.00 c	10.67 c	97	5.60 de
$CIAT_{899}$	22.67 a	22.67 a	100	9.93 bc
F Factor	14.467^{**}	16.216^{**}		14.892^{**}
LSD 5%	4.13	4.06		2.78
LSD 1%	5.68	5.60		3.83
LSD 0.1%	7.82	7.71		5.27

Table 2. Foliar area and accumulated biomass with beans (Aversa cultivar) inoculated with different bacteria strains
Stage : beginning of flowering

Treatment	Foliar area cm^2/pl.	Accumulated biomass (mg d.m./pl.)			
		Roots	Stem	Leaves	Total
Non-inoc. x non-fert.	171.0 d	187.3 bcd	440.7 b	827.3 e	1455.3 d
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$CIAT_{75}$	230.3 b	176.0 cd	534.3 b	929.3 d	1639.7 c
$CIAT_{161}$	240.0 c	194.7 bcd	519.3 b	833.3 e	1547.3 a
$CIAT_{899}$	290.3 a	224.0 ab	724.3 a	1388.7 a	2337.0 a
F value	24.176^{xx}	4.963^{xx}	10.866^{xx}	86.88^{xx}	67.69^{xx}
LSD 5%	25.04	38.40	94.68	68.67	115.23
LSD 1%	34.49	52.90	130.42	94.59	158.72
LSD 0.1%	47.48	72.82	179.54	130.23	218.51

Table 3. Number of nodules on plant and their dry weight with beans (Aversa cultivar) inoculated with different

bacteria strains.
Stage : complete flowering

Treatment	Total nodules nr./pl.	Active nodules nr./pl.	Active nodules %	Dry weight of nodules mg./pl.
Non-inoc. x non-fert.	21.0 e	17.1 c	81	26.7 f
N0n-inoc. x fert.	25.0 d	17.4 c	70	27.0 f
FL_{8}	40.7 bc	30.3 bc	74	40.7 bcd
FL_{23}	30.3 cd	17.0 c	56	29.0 ef
FL_{400}	26.7 d	22.0 c	82	33.0 def
FL_{3644}	43.0 b	27.7 c	64	48.0 b
$CIAT_{75}$	34.0 bcd	22.0 c	65	27.6 f
$CIAT_{161}$	41.0 bc	28.3 bc	69	39.0 be
$CIAT_{899}$	105.7 a	74.3 a	70	60.0 a
F Factor	38.615^{xx}	15.117^{xx}		8.536^{xx}
LSD 5%	11.71	13.50		10.44
LSD 1%	16.14	18.59		14.37
LSD 0.1%	22.21	25.60		19.79

Table 4. Foliar area and accumulated biomass with beans (Aversa cultivar) inoculated with different bacteria strains
Stage : flowering

Treatment	Foliar area cm^2/ pl.	Accumulated biomass (mg d.m. / pl.)			
		Roots	Stem	Leaves	Total
Non-inoc. x non-fert.	468.0 c	0.349 bcc	2.53 cd	2.07 de	4.949 cd
Non-inoc. x fert.	450.7 c	0.356 bc	2.24 d	5.19 a	7.780 a
FL_{8}	804.0 a	0.524 a	4.44 a	3.57 b	8.534 a
FL_{23}	469.7 c	0.301 c	2.19 d	1.94 de	4.437 d
FL_{400}	607.7 b	0.374 bc	3.10 bc	2.40 cd	5.868 bc
FL_{3644}	410.7 c	0.427 b	2.76 cd	1.86 e	5.047 cd
$CIAT_{75}$	552.7 b	0.373 bc	3.43 b	2.75 c	6.556 b
$CIAT_{161}$	440.3 c	0.326 c	2.62 cd	1.83 e	4.779 d
$CIAT_{899}$	554.0 b	0.333 c	2.71 cd	2.30 cde	5.349 cd
F Factor	25.867^{xx}	6.226^{xx}	12.327^{xx}	37.942^{xx}	15.772^{xx}
LSD 5%	71.66	0.08	0.59	0.53	1.07
LSD 1%	98.71	0.11	0.82	0.73	1.48
LSD 0.1%	135.89	0.15	1.13	1.01	2.04

Table 5. Biomass production, nitrogen per cent and total nitrogen quantity with beans (Aversa cultivar) inoculated with different bacteria strains of *Rhizobium* genus.

Stage : maturity

Treatment	Dry biomass (kg/ha)			Yield index %	Nitrogen (%)		Total nitrogen (kg/ha)			Nitrogen yield index %
	Seeds	Second prod.	Total plant		Seeds	Second prod.	Seeds	Second prod.	Total	
Non-inoc. x non-fert.	1755	2295	4050	43.8	3.52	1.18	61.77	27.12	88.89	69.5
Non-inoc. x fert.	2040	2710	4750*	42.9	3.56	1.34	72.62	36.33*	108.95*	66.7
FL_{8}	2295**	2155	4450	51.6	3.42	1.23	78.49*	26.67	105.16*	74.6
FL_{23}	2348**	2437	4785*	49.1	3.50	1.14	82.18**	27.89	110.07**	74.7
FL_{400}	2249*	1931	4180	53.8	3.36	1.33	75.57*	25.80	101.37	74.5
FL_{3644}	2205*	2030	4235	52.1	3.60	1.10	79.38**	22.25	101.63	78.1
$CIAT_{75}$	2266*	1969	4239	53.5	3.60	1.34	81.57**	26.35	107.92*	75.6
$CIAT_{161}$	2040	2085	4125	49.5	3.67	1.49	74.85*	31.10	105.95*	70.6
$CIAT_{899}$	2134	2486	4620	46.2	3.63	1.43	77.46*	35.58*	113.04**	68.5
LSD 5%	388	422	701				12.4	8.30	15.7	
LSD 1%	534	717	823				17.0	12.51	21.6	
LSD 0.1%	744	999	1154				23.8	18.80	30.2	

Table 6. Accumulation of ^{15}N in plants, percentage and quantities of nitrogen derived from fertilization (Ndff) and utilization coefficient of nitrogen from fertilizers with beans (Aversa cultivars) inoculated with different bacteria strains

Treatment	Excess of ^{15}N (%)		NDFF (%)		Ndff (kg/ha)			Utilization coeff.of nitrogen from fertilizer (%)	
	Seeds	Second prod.	Seeds	Total plant	Seeds	Second prod.	Total plant	Seeds	Second prod.
Non-inoc. x non-fert.	-	-	-	-	-	-	-	-	-
Non-inoc. x fert.	0.390	0.342	8.42	7.39	6.11	2.68	8.79	30.6	13.2
FL_8	0.293	0.288	6.32	6.22	4.96	1.65	6.61	24.8	8.3
FL_{23}	0.229	0.236	4.94	5.10	4.05	1.42	5.47^{0}	20.2	7.1
FL_{400}	0.221	0.219	4.77	4.73	3.60	1.22	4.82^{00}	18.0	6.1
FL_{3644}	0.249	0.229	5.37	4.94	4.26	1.10	5.36^{0}	21.3	5.5
$CIAT_{75}$	0.233	0.238	5.03	5.14	4.10	1.35	5.45^{0}	20.5	6.8
$CIAT_{161}$	0.298	0.250	6.44	5.40	4.82	1.68	6.50	24.1	8.4
$CIAT_{899}$	0.205	0.216	4.42	4.66	3.42	1.66	5.08^{00}	17.1	8.3
LSD 5%							1.15		
LSD 1%							1.49		
LSD 0.1%							2.13		

Table 7. Quantity of nitrogen symbiotically fixed in different inoculation treatments according to formula of Handarson and Danso (1993)

Treatment	Biologically fixed N_2						Index of fixed N_2 yield (%)
	Seeds		Second. prod.		Total biomass		
	kg/ha	% of control	kg/ha	% of control	kg/ha	% of control	
Non-inoc. x non-fert.	-	-	-	-	-	-	-
Non-inoc. x fert. (Control)	36.5	100	24.5	100	61.0	100	59.8
FL_8	49.1	135	14.1	56	63.2	104	77.7
FL_{23}	58.8	161^{x}	12.8	52	71.6	117^{x}	82.1
FL_{400}	56.4	155^{x}	14.1	58	70.5	116^{x}	80.0
RCR_{3644}	55.0	151^{x}	16.9	69	71.9	118^{x}	76.5
$CIAT_{75}$	54.7	150^{x}	18.1	74	72.8	119^{x}	75.1
$CIAT_{161}$	47.1	129	20.9	85	68.0	111	69.3

$CIAT_{899}$	57.9	159[x]	19.4	79	77.3	127[x]	74.9
LSD 5%	13.7	37.5	9.4	38.4	9.5	16.1	
LSD 1%	22.8	62.5	12.8	52.2	22.0	36.0	
LSD 0.1%	35.6	97.5	17.9	73.0	31.1	50.9	