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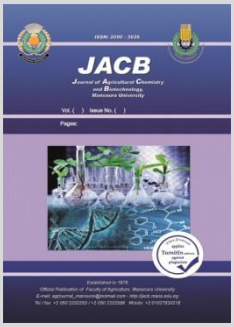
Recombinants Rhizobacteria Enhanced Biological Control of *Rhizoctonia solani* infected Faba Bean

Mervat I. Kamal[1]*; K. A. Zaied[1]; A. H. Abd El-Hadi[1]; Fayza K. Abd El-Fatah[2] and Abeer A. Shawaf [2]

[1] Department of Genetics , Faculty of Agriculture , Mansoura University.
[2] Water, Soil and Environmental Res. Institute, Agric. Res. Center, Giza, Egypt.



ABSTRACT

This study aimed to test the effect of *Rhizobium* recombinants on the vegetative growth and yield components of faba bean under biotic stress of *Rhizoctonia solani*. Tweleve transconjugants resulted from six crosses were evaluated for their antigonism against *Rhizoctonia solani*. The results showed variations in chitin hydrolysis after genome shortening. At the molecular level, the recipient strains of rhizobia showed more similarity with each other. However, the donar strains of *Serratia* and *pseudomonas* showed less similarity. Meanwhile , *Rhizobium* transconjugants genomes have a similar distribution of bands. Both transconjugants Tr $_4$ and Tr$_{15}$ showed more genome size than the other ones due to high level of plasmid transfer. The same transconjugants recorded higher values of chitin hydrolysis zone due to higher expression of chitinase genes. Most transconjugants showed significant increase in nodulation above the uninoculated plants grown in the infected soil. *Rhizobium* inoculants improved the yield , chlorophyll concentration and NPK content of the plants grown in the soil infected with *Rhizoctonia solani* compared with uninoculated plants. The results indicated that *Rhizobium* inoculants improved the growth and yield of plants grown under biotic stress condition. In addition, improved rhizobia with chitinolytic enzymes producing genes could inhibit significantly the growth of pathogenic fungi.

Keywords: *Rhizobium* transconjugants, pathogenic fungi, chitinolytic enzymes, plasmid curing, genome similarity.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important legume crops in Egypt. It is grown mostly for food and feed requirements for human and animal consumption (Morsy and Tarrad, 2005). Nitrogen fixation, one of the most important soil processes, is developed by means of microorganisms. Use of microbial inoculants or plant growth- promoting rhizobacteria (PGPR) for the enhancement of sustainable agricultural production is becoming a more widely accepted practice in intensive agriculture in many parts of the world to avoid environmental pollution resulting from chemical fertilizers (Kumar *et al*. 2014). *Rhizobium* can establish an effective nitrogen-fixing symbiosis through exchanging signal molecules with its plant host .

Root rot, caused by *Aphanomyces euteiches, Rhizoctonia solani, Fusarium spp., Sclerotium rolfsii* is one of the most destructive soil-borne diseases of pea, chickpea, lentil, faba bean and lupine (Infantin *et al.* 2006). Many species of rhizobia promote plant growth and also inhibit the growth of certain pathogenic fungi. Rhizobia are reported to inhibit significantly the growth of pathogenic fungi, *Macrophomina phaseolina* , *Rhizoctonia spp, Fusarium* sp. and *Pythium* spp in both leguminous and non-leguminous plants (Bardin *et al.* 2004). The biocontrol agents have different mechanisms which may be involved in the suppression of different plant diseases. For example, inhibition of the pathogen by antimicrobial substances (antibiosis) such as , parasitism that may involve production of extracellular cell wall-degrading enzymes, such as chitinase that can lyse pathogen cell walls (El-Mehalawy, 2004) . In addition, such agents promote plant growth including better shoot height, root length, dry weight and root nodulation (Siddiqui *et al*. 2000). Furthermore, expression of the *chiA* gene from *S. marcescens* in *R. meliloti* demonstrated that the nodule extracts from *chiA* expressing alfalfa plants caused lysis of *Rhizoctonia solani* hyphal tips (Minic *et al*. 1998). Moreover, *Rhizobium meliloti* expressed *Serratia marcescens* chitinase gene efficiently degrades hyphal tips of *Rhizoctonia solani* (Sitrit *et al*. 1993). Rhizobial species harbours plasmids that vary in size and number. The nodulation genes (*nodABC*), as well as the regulatory (*nodD*) gene and the nitrogen fixing (*nif/fix*) genes are located on large (usually ≥ 100 kb) symbiotic plasmids (pSym) (Mulligan and Long 1985).

Among the genetic methods, DNA macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) has been one of the most successfully adapted technique for a wide range of microorganisms studies (Tenover *et al*. 1995). PFGE profiles use the number of band differences as evidence of strain identification (Struelens and European, 1996).

Pulsed-field gel electrophoresis is one of several tools for separating large DNAs (Schwartz and Cantor 1984). It can be used to investigate global and specific genome organization in species of Rhizobiaceae in a more detailed way than was previously possible (Sobral *et al*. 1991). Many recent studies showed that migration of circular molecules during PFE is different from that of linear molecules (Tenover *et al*. 1995).

Plasmids have been shown to code for the resistance of a variety of antibiotics (Falkow, 1975). In many cases this

* **Corresponding author.**
E-mail address: mervat_y2007@yahoo.com
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resistance is transferable either to different strains of the same species or to different bacterial species (Watanabe, 1963). However, Brom *et al*. (1992) showed that plasmid-curing in *R. leguminosarum* and *R. etli* appeared to significantly reduce their ability to compete with the wild-type because all cured isolates had lost their antibiotic resistance (Abdel-Salam *et al*. 2007).

This investigation aimed to improve growth and yield of Faba bean via biological control of *Rhizoctonia solani* with *Rhizobium* recombinants, as well as, the effect of genome shortening on chitinase degrading cell wall of fungi.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Bacterial strains and their transconjugants used in this study are listed in Tables (1 and 2) which include their source or references, as well as, their designation.

Table 1. Bacterial strains used in this study

Strains	Source or reference	Designation
Rhizobium leguminosarum (3841)	Prof J P W Young, Department of Biology, University of Yourk, UK.	RL-3841
Rhizobium leguminosarum (ARC 207)	Agric. Res. Center, Dept. of Microbiology, Giza, Egypt.	Rl-207
Rhizobium leguminosarum (USDA2074)	Dr. Peter van Berkum, Microbiologyist , National *Rhizobium* culture collection, USDA, Baltimore Avenue Beltsville	RL-2074
Rhizobium leguminosarum (12612)	IAM Culture Collection, Univ. of Tokyo, Japan.	RL- 12612
Pseudomonas fluorescences (NRRL B-23932)	National center for Agriculture Utilization Research, USA.	Pf
Serratia marcescens	Microbiology Dept., Soil, Water and Environmental Research Institute, Agricultural Research Center (ARC).	*Sm*
Rhizoctonia solani	Plant Pathology Institute, Agricultural Research Center (ARC) Giza, Egypt.	*R. solani*

Table 2 . Transconjugants used in this study

Transconjugant designation	Genotype	Source or reference
Tr_1 Tr_2	Nm^+ Cm^+	*Sm* X *RL-207*
Tr_3 Tr_4	Tc^+ Nm^+	*Sm* X *RL-2074*
Tr_5 Tr_6	Tc^+ Nm^+	*Sm* X *RL-12612*
Tr_7 Tr_8	Tc^+ $Stre^+$	*Sm* X *RL-3841*
Tr_9 Tr_{10}	Cm^+ Rif^+	*PF* X *RL-2074*
Tr_{11} Tr_{12}	Cm^+ Rif^+	*PF* X *RL-12612*

Seeds

Faba bean (*Vicia faba* L.) seeds variety Sakha 1 used in this study were obtained from Crops Research Institute, Agriculture Research Centre, Ministry of Agriculture, Giza. The seeds were surface sterilized for 1 min with 70% ethanol, rinsed five times with sterile distilled water and then sterilized again with 0.5% sodium hypochloride according to Asaka and Shoda (1996).

Media and growth conditions

Yeast extract mannitol medium (YEM) : It was used in this study according to Vincent (1970). The strains were maintained and grown at 28 ^{0}C in YEM . However, the minimal medium was used for preparing isolates and strains for electrophoretic analysis according to Balassa (1963). In addition, TY Medium was used in curing experiments according to Beringer (1974).

Pots experiment

Pots experiment was conducted during the winter Faba bean growing season of 2014-2015. Plant infection technique was commonly applied in this study to determine the efficiency of different transconjugants in symbiosis according to Vincent (1970).

Preparation of pathogenic fungal inoculum

Rhizoctonia solani was grown on autoclaved sorghum sand medium for 15 days at 25^{0}C . It was inoculated into the soil five days before sowing at the rate of 3g / kg soil (Hassanein *et al.* 1997).

Nodulation test

After 55 days of inoculation nodules were counted, dried and weighted according to Novak *et al*. (2004).

Average weight of nodule (AWON)

It was estimated according to Pereira *et al*. (1989).

Photosynthetic pigments

Chlorophyll contents (a , b and total chlorophyll) were extracted in 80% methanol. The pigments were determined spectrophotometrically after the extracted solution was stored for twenty four hours in a refrigerator. Chlorophyll concentrations were calculated according to Lichtenthaler and Wellburn (1983).

Plant mineral content

Samples of roots, shoots and seeds were dried and powdered to determine total NPK. Determination of total N was done by kjeldahl's method, while the total P content was measured by vanado – molybdate yellow color method (Papakosta and Gagianas, 1991). Total K^+ was measured by flame photometric method (Ryan *et al*., 2001).

Plasmid Curing

To determine the resistance of antibiotic encoded by a plasmid or chromosomal genes, strains were subjected to elevated temperature up to 40 ^{0}C. Culturs were inoculated into TY broth medium and incubated at 40 ^{0}C for 3 h and then plated on TY medium containing 5% sucrose. The plates were incubated at 28°C for 3 days. Single colonies were picked up and rechecked for the same antibiotic resistance pattern to ensure the stability of resistance obtained before (Bastos *et al*. 1980).

Isolation of genomic DNA

Genomic DNA was extracted from bacterial strains and their transconjugants were grown in 100 ml minimal medium at 30°C overnight and quantify at 1.0 % agarose by agarose gel electrophoresis. Agarose was supplemented with 0.5 µg/ml ethidium bromide stain. Furthermore, five ml of the extracted DNA was loaded in agarose gel according to Barzegari *et al.* (2010). After the electrophoresis was complete the gel was observed via Gel Documentation System under UV light according to Atashpaz *et al.* (2010).

Statistical analysis

Data were subjected to the analysis of variance according to Snedecor and Cochran (1955). Least significant difference (L.S.D.) was used to compare between means .

RESULTS AND DISCUSSION

Genome shortening

Biological control is an alternative approach of reducing chemical pesticides in agricultural practices. On the

other hand, chitin is the second component of biomass in nature after cellulose and can be hydrolysed to monomers by chitinase. Plasmid curing conducted in this study was performed to locate chitinase properties on plasmids or bacterial chromosome. Table (3) shows the results of eleven cured colonies which were still showing the same chitinase activity as the original strain. In addition , six cured colonies lost chitinase activity. There was variations in the ability of bacterial strains and their recombinants after plasmid curing in chitin hydrolysis to form inhibition zone. The results indicated that chitinase genes may be chromosomally located in most cases. However, in some strains these genes were plasmid located. This agreed with Zhong *et al*. (2015), who isolated chitinase C gene from chromosomal DNA of *Pseudomonas sp.* using a pair of specific primers. This gene plays an important role in chitin metabolism to be used as a carbon and energy source by microorganisms. In addition, Sridevi and Mallaiah (2008) found that out of 26 *Rhizobium* strains isolated from root nodules of *Sesbania sesban*, only 12 strains showed chitinase activity. The same authors also found that *Rhizobium sp.* could degrade the mycelia of *Aspergillus* and *Fusarium.*

Table 3. Effect of high temperature on plasmid curing

Bacterial strains	Antibiotic resistance at 28°C	Antibiotic resistance after curing at 40°C	Chitin analysis before plasmid curing	Chitin analysis after plasmid curing
Sm	TC^+ Cm^+	TC^+ Cm^+	1.6	1.6
Pf	Rif^+	Rif^-	1.5	1.6
RL-207	Nm^+	Nm^+	1.0	1.0
RL-2074	Nm^+ Cm^+	Nm^- Cm^-	0.0	0.0
RL-12612	Nm^+ Cm^+	Nm^- Cm^-	1.5	0.0
RL-3841	$Strep^+$	$Strep^+$	1.6	1.6
Tr_1	Nm^+ Cm^+	Nm^+ Cm^+	1.8	1.8
Tr_2	Nm^+ Cm^+	Nm^- Cm^-	2.3	0.0
Tr_3	TC^+ Nm^+	TC^- Nm^-	2.1	0.0
Tr_4	TC^+ Nm^+	TC^- Nm^-	1.8	0.0
Tr_5	TC^+ Nm^+	TC^- Nm^-	2.2	2.3
Tr_6	TC^+ Nm^+	TC^- Nm^-	1.4	1.5
Tr_7	TC^+ $Strep^+$	TC^- $Strep^-$	2.1	0.0
Tr_8	TC^+ $Strep^+$	TC^+ $Strep^+$	2.0	2.0
Tr_9	Cm^+ Rif^+	Cm^- Rif^-	1.1	0.0
Tr_{10}	Cm^+ Rif^+	Cm^+ Rif^+	1.7	1.7
Tr_{11}	Cm^+ Rif^+	Cm^+ Rif^+	1.6	1.6
Tr_{12}	Cm^+ Rif^+	Cm^- Rif^-	1.6	1.7

Recombinant rhizobia harboring chitinase genes are receiving much attention as a biological control agent against fungi. This agreed with Sitrit *et al.*(1993), who reported that *Rhizobium meliloti* with expressed *Serratia marcescens* chitinase gene efficiently degrades hyphal tips of *Rhizoctonia solani.* Plant symbiotic bacteria are potential agents for controlling plant diseases and also as excellent root colonizers which suggests their use as biocontrol agents. Nahar *et al.* (2012) found that rhizobial strains were found to be sensitive against some antibiotics after plasmid curing and no exopolysaccharides were found. This indicated that plasmid curing decreased symbiotic nitrogen fixation and probably chitinase activity, suggesting a link between these properties and plasmids.

Characterization of *Rhizobium* transconjugants at the molecular level

Pulsed-field gel electrophoresis (PFGE) was used in this study for genetic characterization of *Rhizobium* strains and their transconjugants to determine genomic fingerprinting of the isolates. This technique offer resolution of the large fragments of genomic DNA resulted by rare-cutting restriction enzymes and the resulting total DNA profiles were dependent on the infrequently occurring recognition sites (Bustamante *et al*. 1993). PFGE was used to show genomic fingerprint patterns of two donar strains against four recipient strains of *Rhizobium* and their transconjugants (Sobral *et al.*1991) .

As shown in Figure (1) *Serratia marcescence* and *Pseudomonas flurocences* donor strains showed less similarity as some bands appeared in *Serratia* disappeared in *Pseudomonas*. In addition, four recipient strains of *Rhizobium* showed more similarity with each other in the number of bands, as well as, their intensity. This analysis suggested that there is a high level of agreement among the genome size of *Rhizobium leguminosarum* strains obtained from different sources.

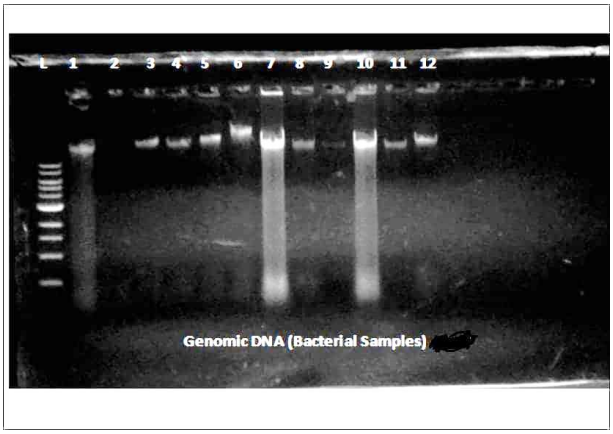

Figure 1. Gel electrophoresis of genomic DNA extracted from bacterial strains. Lane: 1 to 2 (*Serratia marcescence* DNA and *Pseudomonas flurocences* DNA). lane 3-6 *Rhizobium leguminosarum* DNA and lane 7 to 12 transconjugants .

In addition , the DNA fragments generated through digestion the genome of four *Rhizobium* strains may be similar in their sizes. Meanwhile, most of *Rhizobium* transconjugants genomes appeared to have a similar distribution of bands, except transconjugants in the lane number 9 which was similar to the donor strain *Pseudomonas flurocences* in the number of bands but each other was differed in the intensity of the bands. On the other hand , both transconjugants in the lanes number 7 and 10 have similar distribution of bands and of their intensity. These transconjugants were genetically differed from the other transconjugants as well as their parents.

Rhizobium transconjugants showed high level of similarity with their parents as they were generated from a common ancestor, *Rhizobium leguminosarum*. Therefore, such rules must ideally be used to indicate strain differences not only strain identity. However, in application it is important to have criteria which can provide a high degree of probability that the transconjugants differed from each other and with the parental strains.

The results obtained in this study are in harmony with Tenover *et al*. (1995), who demonstrated that the interpretation of molecular fingerprints are dependent on the number of genetic differences generated by any genotype in a putative outbreak compared with the most common profile. One genetic event resulted from three band differences and the isolate would be considered to be closely related to the outbreak strain and became part of the outbreak. The results obtained in this study agreed with this idea and indicated that

Rhizobium transconjugants are part of the outbreak of the parental strains used herein.

However, two genetic events can generate up to six bands differences and the isolates may be possible part of the outbreak. Meanwhile, three or more independent genetic events would produce seven or more band differences, such isolates would not be considered as part of the outbreak. The PFGE results appeared in this study could be used to assign genotype with confidence particularly to isolates which show equivocal phenotypic typing patterns. However, similarity was shown among recipient strains of *Rhizobium*, this may be due to the fact that they are of the same wild genotype which may display distinct profiles.

This could reveal why macrorestriction analysis of DNA by PFGE is an important technique for typing bacterial strains and it was a powerful discriminatory potential for the characterization of isolates (Johnson *et al*. 2007). The results revealed that banding patterns were intense, clear and reproducible to apply on bacterial strains and their transconjugants as a key for molecular characterization of studied bacteria.

The DNA patterns in lane number 7 (Tr_4) and lane number 10 (Tr_{15}) showed more genome size than other transconjugants as well as than the parental strains. Therefore, these transconjugants could show genetic diversity from different transconjugants and their original parents which could be due to genome increase because of plasmid transfer from the donar to the recipient strains. In fact, both transconjugants showed higher diameter of clear zones related to chitinase production.

Taken together, high expression of chitinase genes may be related with the genome size which provide valuable information about hydrolyzing activity of chitinase in bacterial isolates. More definitive results about hydrolyze activity of chitinase will be obtained from bioactivity assay of each genotype because a discrepancy exists between chitinase gene and its antifungal activity. These results showed that there was a relation between PFGE patterns and chitinase activity as shown in the lanes numbered 7 and 10. Interestingly, the transconjugants generated in this study differed in the sizes of their entire genome and in the expression of chitinase genes which reflect phenotypic variability in chitin hydrolysis.

Nodulation

The data summarized in Table (4) showed that most of biofertilizer inoculants revealed significant positive effect on nodulation parameters. Meanwhile, most transconjugants showed significant increase in nodulation above the uninoculated plants grown in infected soil. This indicated that rhizobial transconjugants may suppress the growth of *Rhizoctonia solani* compared with the parental strains. The suppression induced by transconjugants against fungi may be due to cell wall degrading enzyme produced by transconjugants. These results agreed with Kumar *et al*. (2001) who found that rhizobia inhibited mycelial growth of plant pathogens such as *Aphanomyces euteiches, Phoma medicaginis, Macrophomina phaseolina, Rhizoctonia solani, Phytophthora cactorum* and *Fusarium spp*. Moreover, Jensen *et al*. (2002) found that *Rhizobium* inoculation effectively suppressed diseases caused by *F. solani, Fusarium oxysporum, Rhizoctonia bataticola and Pythium sp*. in soil naturally infested with these pathogens. However, Huang and Erickson (2007) found that pea seeds treated with *R. leguminosarum bv. viceae* resulted in significant ($P < 0.05$) reduction in damping-off compared with the untreated control. Rakesh *et al*. (2004) found that application of fungi alone or combined with rhizobia reduced disease incidence of some soil-born disease. Furthermor, Bhuiyan *et al*. (1998) found that *Rhizobium* inoculation increased nodulation and seed yields up to 35%. In addition, Bejandi *et al*. (2012) found that seed inoculation with *Rhizobium cicerea* produced significantly highest nodule number, nodule fresh weight, nodule dry weight and active nodule per plant than uninoculated control.

Table 4. Nodulation parameters in faba bean plants inoculated with bacterial strains and their transconjugants

Inoculants	Nodules developed /plant		Nodule DW (mg/plant)		Average weight of nodule (mg)		Nodulation index	
	I	II	I	II	I	II	I	II
Uninoculated	1	0	1	1	1	1	0.17	0.2
Sm	2	1	3	2	1.5	1	0.31	0.32
PF	1	1	9	1	9	1	1.15	0.13
RL-207	99	13	176	28	1.77	2.15	10.60	4.30
RL-2074	43	7	157	12	3.65	1.71	10.61	2.67
RL-12612	20	6	135	9	6.75	1.5	11.54	1.53
RL-3841	49	4	213	6	4.35	1.5	33.28	0.98
Tr_1	26	12	116	20	4.46	1.67	14.68	1.68
Tr_2	16	9	108	19	6.75	2.11	6.51	1.28
Tr_3	8	49	16	110	2	2.24	1.28	5.67
Tr_4	15	54	294	353	19.6	6.54	22.27	55.16
Tr_5	11	34	22	71	2	2.09	2.26	15.43
Tr_6	6	18	13	36	2.17	2	1.19	4.44
Tr_7	74	75	98	146	1.32	1.94	6.04	11.06
Tr_8	51	22	85	179	1.67	8.14	6.20	8.65
Tr_9	55	12	114	23	2.07	1.92	10	3.33
Tr_{10}	21	24	31	163	1.48	6.79	2.46	14.55
Tr_{11}	26	29	222	97	8.54	3.34	20	7.23
Tr_{12}	18	24	87	184	4.83	7.67	5.72	16.58
F-test	**	**	IS	**	**	**	**	**
LSD 0.05	14.9	17.1		6.05	2.29	1.7	3.25	7.4
LSD 0.01	19.9	22.8		8.11	3.07	2.28	4.36	9.8

I , II : uninfected and infected soil , respectively.
IS : Insignificant differences.
****: significant at 0.05 and 0.01 probability level, respectively.**

Yield components

Table (5) showed that yield and yield components were significantly increased in response to inoculation with bacterial strains and their transconjugants in relation to uninoculated plants.

Table 5 . Yield and yield component traits of faba bean plants inoculated with bacterial strains and their transconjugants.

Inoculants	Number of pods/plant		Number of seeds/pod		Seeds dry weight (g/plant)	
	I	II	I	II	I	II
Uninoculated	3	1	2	1	5.0	2.0
Sm	4	2	2	3	3.0	6.1
PF	3	2	3	2	2.8	4.3
RL-207	8	4	5	3	32.6	16.0
RL-2074	7	3	4	3	32.0	9.8
RL-12612	7	6	5	3	25.0	14.2
RL-3841	6	3	4	3	12.3	11.8
Tr_1	6	7	4	4	17.2	26.8
Tr_2	7	8	3	5	17.6	26.8
Tr_3	7	9	4	5	21.7	35.6
Tr_4	7	6	5	4	23.0	22.7
Tr_5	6	7	4	5	19.2	22.8
Tr_6	9	7	4	4	22.0	22.6
Tr_7	8	9	5	4	26.6	36.6
Tr_8	7	7	4	4	29.1	37.1
Tr_9	7	7	4	4	25.7	26.3
Tr_{10}	6	9	4	4	25.6	24.4
Tr_{11}	5	6	4	5	15.5	22.4
Tr_{12}	4	7	4	4	14.5	23.4
F-test	**	**	**	**	**	**
LSD 0.05	2.2	2.2	1.2	1.1	6.4	7.4
LSD 0.01	3.0	2.8	1.6	1.5	8.5	9.8

I , II : Uninfected and infected soil , respectively.
****: significant at 0.05 and 0.01 probability level, respectively**

On the other hand, *Rhizobium* inoculants improved the yield in fungal infected soil if compared with that in uninfected soil. Significant increase in yield components may be due to growth hormones, nitrogen fixation and antagonism with

pathogenic fungi were inhibited by rhizobial inoculants. These results are in harmony with Chernin *et al*. (1955) ,who found that many species of bacteria produce chitinolytic enzymes. In addition, Jensen *et al*. (2002) found that rhizobia inhibit significantly the growth of pathogenic fungi such as, *Rhizoctonia spp, Fusarium sp. and Pythium spp*. in both leguminous non-leguminous plants. Huang and Erickson (2007) showd that seed treatment with *R. leguminosarum bv viceae* was effective in controlling damping-off and promoted growth of pea and lentil plants grown in naturally infested with *Pythium spp*. Morever , Khosravi *et al*. (2001) showed that faba bean seeds inoculated with some native *Rhizobium leguminosarum bv. viciae* increased seed yield from 35% to 69% due to the inoculation. Denton *et al*. (2013) suggested that rhizobial inoculation increased shoot growth, number of pods and grain yield of faba bean. Namvar *et al*. (2011) found that plants inoculated with *Rhizobium* recorded about 8.00% more pods per plant than non-inoculated plant.

Biochemical traits

Table (6) showed significant differences between treatments in chlorophyll a , b and total in the infected soil .In contrast, uninfected soil showed insignificant differences between inoculants, except for total chlorophyll which showed significant differences between inoculants . In addition , some of transcojugants such as ; Tr_2 , Tr_3 , Tr_8 and Tr_{11} showed significant increase in chlorophyll a , chlorophyll b , as well as, total chlorophyll in relation to uninoculated plants. These results agreed with Bambara and Ndakidemi (2009),who found that leaf chlorophyll (Chl) content increased significantly with rhizobial inoculation. In addition, Safikhani *et al*.(2013) found the highest total chlorophyll content (1.3 mg/g) in plants inoculated by *Rhizobium*. On the other hand, Katundala (2011) found that the early vegetative growth stage of soybean plant and leaf chlorophyll content increased following inoculation while at the late pod filling stage shows just a significant effect on the leaf chlorophyll content following inoculation if compared with uninoculated plants. However, Peng *et al*. (2002) found that the growth promotion by rhizobial inoculation could be attributed to the increase in photosynthetic rate.

Table 6 . Leaves chlorophyll content of plants inoculated with bacterial strains and their transconjugants.

Inoculants	Chlorophyll content (mg/g)					
	Chlorophyll a		Chlorophyll b		Total chlorophyll	
	I	II	I	II	I	II
Uninoculated	0.96	0.271	6.33	1.284	7.29	1.55
Sm	1.94	0.269	5.80	1.21	7.75	1.497
PF	1.19	0.899	5.90	3.21	7.09	4.099
RL-207	1.42	0.930	5.75	1.014	7.17	2.07
RL-2074	1.68	0.180	3.98	1.94	5.66	19.94
RL-12612	1.17	1.28	6.04	2.78	7.20	4.06
RL-3841	1.96	1.43	5.93	1.542	7.89	2.972
Tr_1	5.89	4.37	5.85	1.588	11.74	5.958
Tr_2	2.67	2.25	11.55	9.13	14.22	11.38
Tr_3	3.78	3.93	11.66	10.51	15.44	14.44
Tr_4	4.38	2.81	9.89	7.12	14.27	9.93
Tr_5	1.73	1.30	5.84	6.79	7.58	8.09
Tr_6	5.92	2.14	3.35	2.99	9.27	5.13
Tr_7	3.10	2.89	5.95	3.11	9.05	6.00
Tr_8	3.09	3.27	9.01	8.57	12.11	11.84
Tr_9	7.13	5.51	9.72	7.01	16.85	12.52
Tr_{10}	4.30	3.56	7.96	7.10	12.26	10.66
Tr_{11}	4.82	2.54	7.64	8.09	12.45	10.63
Tr_{12}	5.99	4.79	5.67	6.90	11.66	11.69
F-test	IS	**	IS	**	**	**
LSD 0.05		1.28		6.03	5.86	3.33
LSD 0.01		1.71		8.08	7.83	4.64

I , II : Uninfected and infected soil , respectively.

S : Insignificant differences.

****: significant at 0.05 and 0.01 probability level, respectively.**

Some bacterial strains , as well as most of *Rhizobium* transconjugants showed significant increase in NPK content in relation to uninoculated plants Table (7). In addition, the efficient transconjugants positively affecting NPK were Tr3 , Tr7 and Tr 8. This agreed with Singh *et al*. (2010) who reported that N content of shoot in the single application of *Rhizobium leguminosorum* was higher than those in single or dual applications of the *Arbuscular mycorrhizal* fungi . In addition , Gabr *et al*. (2007) showed that inoculation of pea seeds with different biofertilizer types increased significantly N, K and Chlorophyll contents of leaves as compared with uninoculated treatment. Meanwhile, Clayton *et al*. (2004) found that *Rhizobium* inoculant increased plant nitrogen content from 19/42 mg plant-1 , while increasing percentage of N derived from air (Ndfa) from 10 to 61% on pea plant.

Table 7. NPK content in faba bean plants inoculated with bacterial strains and their transconjugants.

Inoculants	Nitrogen		Phosphorus		Potassium	
	I	II	I	II	I	II
Uninoculated	0.69	0.05	1.4	0.05	1.70	1.60
Sm	0.80	0.13	1.27	1.27	1.70	1.72
PF	0.56	0.57	0.0	0.47	0.49	0.83
RL-207	1.47	1.21	0.28	0.20	2.45	2.13
RL-2074	0.17	2.17	0.22	0.08	2.50	1.70
RL-12612	1.88	2.06	0.44	0.25	2.72	2.07
RL-3841	0.25	0.543	0.27	0.21	2.98	1.65
Tr_1	2.87	2.27	0.35	0.37	2.56	2.97
Tr_2	2.31	1.24	0.71	0.36	2.35	2.30
Tr_3	3.93	2.55	2.67	2.37	2.55	2.34
Tr_4	2.90	3.3	2.78	2.80	2.05	2.26
Tr_5	2.82	1.13	0.12	0.12	2.53	2.13
Tr_6	1.16	2.99	0.12	0.13	1.38	0.56
Tr_7	3.26	2.23	2.67	2.06	2.91	2.56
Tr_8	2.40	2.76	2.93	2.66	1.81	2.83
Tr_9	2.13	2.2	2.49	1.23	3.00	1.58
Tr_{10}	2.47	1.2	2.16	1.80	1.77	1.98
Tr_{11}	2.54	2.53	2.47	2.53	2.22	1.32
Tr_{12}	2.88	3.5	1.67	2.65	1.88	1.43
F-test	**	**	**	**	**	**
LSD 0.05	0.37	1.20	1.65	0.44	0.55	0.68
LSD 0.01	0.49	1.61	2.21	0.59	0.74	0.92

I , II : Uninfected and infected soil , respectively.

****: significant at 0.05 and 0.01 probability level, respectively.**

In conclusion, recombinant isolates of rhizobia harboring chitinase genes had a positively significant effect on the vegetative growth , as well as , biochemical traits and yield components of faba bean under biotic stress of *Rhizoctonia solani*. Meanwhile, the results after genome shortening showed variations in chitin hydrolysis. In addition, pulsed-field gel electrophoresis used in this study demonstrated that the DNA analysis were effective in detecting genetic variability between *Rhizobium* strains and their transconjugants .

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تحسين المقاومة الحيوية لفطر الريزوكتونيا سولاني الذى يصيب الفول البلدى بإستخدام إتحادات وراثية جديدة من الريزوبكتيريا

ميرفت إبراهيم كمال [١] ، خليفه عبد المقصود زايد[١] ، أشرف حسين عبد الهادى[١] ، فايزة كمال عبد الفتاح [٢] و عبير أحمد شواف[٢]

[١] قسم الوراثة – كلية الزراعة – جامعة المنصورة

[٢] معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية بالجيزة

تهدف هذه الدراسة إلى إختبار تأثير الإتحادات الوراثية الجديدة من ريزوبيا الفول البلدى على صفات النمو الخضري والمحصول في نباتات الفول تحت ظروف العدوى الصناعية بفطر الريزوكتونيا سولاني. تم تقييم التضاد الميكروبى ضد فطر الريزوكتونيا سولاني بإستخدام ١٢متحولة تزاوجية تم عزلهم من ست تهجينات مختلفة. أوضحت النتائج وجود إختلافات في تحليل الشيتين بعد إختزال حجم الجينوم. أظهرت سلالات الريزوبيا المستقبلة للمادة الوراثية تشابه كبير بين كل منهما والأخرى على المستوى الجزيئي. بينما أظهرت السلالات المعطية للمادة الوراثية فى عملية التزاوج وهى السراتيا والسيدوموناس درجة أقل من التشابه فيما بينهما. بالإضافة إلى ذلك ، فإن جينوم المتحولات التزاوجية للريزوبيا قد أظهر تشابها فى توزيع حزم المادة الوراثية على الجيل. كما أظهرت المتحولات التزاوجية Tr_4 ، Tr_{15}, حجم أكبر للجينوم بالمقارنة ببباقى التراكيب الوراثية الجديدة الأخرى ويرجع ذلك إلى معدل النقل البلازميدى المرتفع من السلالات المعطية إلى هذه التراكيب الوراثية. أظهرت نفس المتحولات التزاوجية ذات حجم الجينوم المرتفع معدلات مرتفعة في إنتاج إنزيم الشيتينيز الناتج عن التعبير الجينى لإنتاج الإنزيم. أظهرت معظم المتحولات التزاوجية زيادة معنوية في صفات تكوين العقد الجذرية تفوق النباتات الغير ملقحة بالريزوبيا في التربة المعداه بالفطر. أدت لقاحات الريزوبيا إلى تحسين صفات المحصول ، تركيز الكلوروفيل في الأوراق ، محتوى النبات من النيتروجين والفوسفور والبوتاسيوم وذلك بالنسبة للنباتات النامية في التربة المعداه بالفطر مقارنة بالنباتات الغير ملقحة بالريزوبيا. تعكس النتائج المتحصل عليها من هذه الدراسة أن لقاحات الريزوبيا تعمل على تحسين صفات النمو والمحصول فى النباتات النامية تحت ظروف الإجهادات البيئية الحيوية. هذا بالإضافة إلى أن تحسين سلالات الريزوبيا بالجينات المنتجة لإنزيم الشيتينيز سوف تثبط بشكل جوهرى من نمو الفطريات الممرضة للنباتات.