Defence Life Science Journal, Vol. 5, No. 1, January 2020, pp. 45-48, DOI : 10.14429/dlsj.5.15119

Effect of Compatible and Incompatible Endophytic Bacteria on Growth of Chickpea Plant

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ABSTRACT

Chickpea is one of the important pulse crops among legumes due to its high protein content. During the last few decades chickpea production has declined because of various biotic and abiotic factors. To increase its production farmers are relying on the traditional methods (using chemical fertilizers) that pollute the environment. An alternative to chemical fertilizers is the eco-friendly process of endophytic inoculation. Compatible endophytic coinoculations improve plant growth as compared to single inoculation due to the synergistic performance of the constituent bacteria. In the current study, the compatibility of six bacterial inoculants (BM5 (rhizobial), BP2 and P36 (phosphate solubiliser), RE2, HE8, and ME9 (other endophytes) was tested. Among these bacterial inoculants, endophyte ME9 was found to be compatible with phosphate solubilising bacteria (P36) and rhizobial culture BM5. However, the endophytic bacteria RE2 and HE8 were found to be incompatible with phosphate solubilising bacteria and rhizobial bacteria. Further, individual inoculation, combined compatible and combined incompatible inoculants were applied to chickpea seeds in the pot house experiment. The results revealed that among all the inoculations, compatible bacterial consortia (ME9, P36 and BM5) produced highest increase in shoot (225%) and root dry weight (600 %) and grain weight (250 %) compared to the control group. The incompatible inoculations were ineffective in improving the root dry weight, shoot dry weight, and grain weight in comparison to the respective individual inoculations.

Keywords: Compatible; Incompatible; Endophyte; Inoculations

1. INTRODUCTION

Chickpea, being rich in protein content, is an important legume crop[1]. Traditional methods of increasing the yield of the chickpea crop involve an excessive use of chemical fertilizers which are hazardous to the environment. Endophytic bacterial inoculation is seen as an environment friendly as well as sustainable alternative for fertilizers to promote the growth of chickpea crops. Endophytes are bacteria that live inside plant tissue and can improve plant growth[2,3]. Endophytic bacteria can directly improve plant growth in multiple ways; including but not limited to nitrogen fixation, phytohormone production and phosphate solubilisation. Also, the introduction of endophytes improves the production of siderophores, that protect the plants from various pathogenic bacteria[4]. Some examples of plant growth promoting bacteria include *Bacillus, Pseudomonas, Enterobacter, Rhizobium, Sinorhizobium* and *Gluconobacter*[5,6]. The use of endophytic inoculants is a low cost and sustainable solution for improving crop production and to meet the food requirements of a growing population.

The inoculation of plants with useful bacteria offers benefits by increasing the plant growth and enhancing plant immunity[7,8] . It has been argued that inoculation of seeds with a variety of beneficial bacteria can further improve plant growth in comparison to inoculation with single type of bacteria[9]. This is due to the synergistic interaction between compatible bacterial consortia that help in promoting plant growth. Some examples include the coinoculation of chickpea with *Mesorhizobium*, *Serratia marcescence*, *Serratia* spp. These have been observed to improve the chickpea plant growth, measured in terms of nodule dry weight, nodule number, number of pods per plant, grain yield, protein content, and total chlorophyll content, as compared to inoculation with a single bacterium in the irrigated, as well as rain fed conditions[10]. Inoculating a consortium of five diazotropic bacteria (*Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Azospirillum amazonense*, and *Paraburkholderia tropica*) reported to have resulted in a higher stem production in sugarcane plants, as compared to single bacterial inoculated plants[11]. However, incompatible bacterial consortia can potentially interact antagonistically, and may even damage the crop. Sugarcane plants (variety SP70-1143) coinoculated with a mixture of *Herbaspirillum* (*H. seropedicae* and *H. rubrisubalbicans*), a consortium, whose constituents showed antagonistic behaviour with each other, resulted in no gains for the overall yield of sugarcane[11]. Therefore, it is important to examine the compatibility of different bacteria in a consortium, prior to its field trial. Consequently, in this study, we first checked the

Received : 23 September 2019, Revised : 03 February 2020
Accepted : 06 February 2020, Online published : 19 February 2020

compatibility between different endophytic cultures used, and then applied the bacterial consortium to chickpea plant seeds to study their effect in the pot house experiments.

2. MATERIALS AND METHODS

2.1 Bacterial Culture

Six bacterial isolates, isolated previously, were used to study plant growth promotion of chickpea plant. Among these six bacterial isolates, one was identified as rhizobium (BM5), two were phosphate solubiliser (P36 reference strain, and BP2) and three were categorised as other endophytes (HE8, ME9 and RE2) on the basis of their isolation process on the different media plates. A YEMA medium was used for rhizobial isolation, a phosphate solubilising medium was used for phosphate solubilising bacteria and Tryptone soya agar was used for other endophytes. The presence of Rhizobium (BM5) was again confirmed using its nodule forming capability under sterilised conditions.

2.2 Compatibility Test between Bacterial Strains

In order to apply the consortium to seeds of chickpea plant for increasing growth, compatibility between the bacterial culture of the consortium was tested by spreading one bacterial culture on the respective media plates, while other cultures were spotted on the same plate. If a zone of inhibition was observed, then the bacterial cultures were concluded to be incompatible. On the other hand, in the absence of inhibition zone, the bacterial cultures were taken to be compatible.

2.3 Individual and Combined Inoculation Studies under Pot House Conditions

Pot house experiment was conducted to investigate the effect of individual and combined inoculation of bacterial endophytes on the chickpea plant growth. Five kg of soil was used in each earthen pot. All the seeds were sterilised by using 0.1 % $HgCl_2$ and alcohol and were inoculated with one ml bacterial inoculum (10^8 CFU/ml) of log phase culture. One control treatment was also kept without any bacterial treatment. These experiments were conducted at CCS-HAU, Hisar, Haryana, India the crop was sown in the month of November, and was harvested in March. Three replicates were maintained for each treatment.

The following treatment groups were tested:

i. Control
ii. HE8 (T1)
iii. ME9 (T2)
iv. RE2 (T3)
v. BP2 (T4)
vi. P36 (T5)
vii. BM5 (T6)
viii. HE8+BM5+BP2 (T7)
ix. HE8+BM5+P36 (T8)
x. ME9+BM5+BP2 (T9)
xi. ME9+BM5+P36 (T10)
xii. RE2+BM5+BP2 (T11)
xiii. RE2+BM5+P36 (T12).

At the time of the harvest of the chickpea plants, the following data were recorded

(a) Root dry weight
(b) Shoot dry weight and
(c) Grain weight.

2.4 Statistical Analysis

PrismPad software was used to analyse the data obtained from the harvested plants using ANOVA with Tukey's Multiple comparison test.

3. RESULTS

3.1 Bacterial Strains

Among six bacterial cultures studied, one was a rhizobium (BM5), two were phosphate solubiliser (BP2 and P36), and three were other endophytes (HE8, ME9, RE2). All the six culture showed plant growth promoting characterstics like Indole acetic acid production (IAA) production, phosphate solubilisation and the siderophore production.

3.2 Compatibility between Bacterial Strains

The three other endophytic bacterial cultures were spread on TSA plates (with each culture on separate plate). The rhizobial and phosphate solubilising bacterial cultures were spotted on to the TSA plates, which showed that both HE8 and RE2 culture were noncompatible to phosphate solubilisers and rhizobial culture. Only ME9 bacterial culture was found to be compatible with P36 and BM5 (Table 1). The compatibility tests were performed between other endophtes, and phosphate solubilising bacteria and rhizobial bacteria, in order to achieve a consortium of one endophytic bacterium, one phosphate solubilising bacterium and one rhizobium culture in a combination of three.

Table 1. Compatibility test among endophytic bacteria with rhizobium and phosphate solubiliaing bacteria

Other endophytes and Rhizobium	P-solubilising bacteria	P-solubilising bacteria	Rhizobium
	BP2	P36	BM5
HE8	NC	NC	NC
ME9	NC	C	C
RE2	NC	NC	NC
BM5	C	C	-

NC- Not Compatible, C- Compatible

3.3 Individual and Combined Bacterial Inoculation Studies of Chickpea Plant under Pot House Conditions

In the present study, six bacterial cultures were applied to chickpea seeds individually and in different combinations to study the effect of compatible and incompatible bacteria on the shoot dry weight, root dry weight and grain weight of chickpea plant at the time of harvesting. After conducting the compatibility test, we made consortia of compatible and incompatible bacteria and applied it to seeds of chickpea in a pot house experiment, to know the effect of compatibility and incompatibility of bacterial consortium on chickpea plant growth.

In our study it was observed that among the individual inoculation of bacterial culture to chickpea seed (T1 to T6 treatment), bacterial culture ME9 (T2) showed highest increase in shoot dry weight (2.4 g/plant). In conformity with the compatibility of ME9 to both BM5 and P36 bacterial cultures, our results showed that, treatment T10 (ME9+BM5+P36) produced significantly higher shoot dry weight (3.9 g/plant) among all the treatments. In contrast incompatible consortium treatment T8 (HE8+BM5+P36) showed lowest shoot dry weight (1.43 g/plant) among all the combined inoculations treatment. It was also observed that the combined inoculation treatment T8 (HE8+BM5+P36) led lower value of shoot dry weight (1.43 g/plant) than the individual inoculations (T1) HE8 (2.3 g/plant) and (T6) BM5 (1.9 g/plant) and almost similar shoot dry weight with (T5) P36 (1.42 g/plant) (Fig. 1).

For root dry weight, it was observed in our study that all the treatments showed increase in the root dry weight than the uninoculated control treatment. Among all the treatments including individual as well as combined inoculations, treatment T10 which is a combination of ME9+BM5+P36 showed significantly highest root dry weight (1.54 g/plant) following individual inoculation treatment T2 (0.96 g/plant). Both treatments T2 and T10 showed significantly higher root dry weight than the control treatment and also from each other. It was also observed that root dry weight of the all the combined inoculation treatments, except treatment T10, were lower than the individual inoculation treatment T2 (Fig. 2).

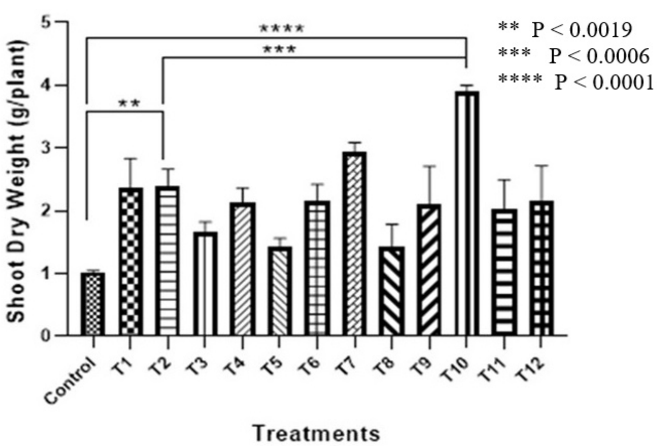

Figure 1. Effect of compatible and incompatible endophytic bacteria on shoot dry weight of chickpea plant.

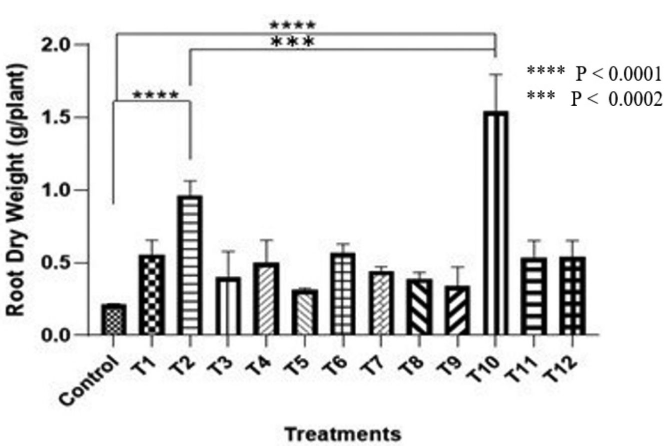

Figure 2. Effect of compatible and incompatible endophytic bacteria on root dry weight of chickpea plant.

A similar trend was observed in the grain weight among individual treatments, treatment (T2) ME9 showed higher grain weight (1.09 g/plant) and among all the treatments, combined treatment T10 showed highest increase in grain weight (1.71 g/plant). It was also observed that the grain weight in THE individual inoculation treatments T1, T2 and T4 were almost same and higher than the all combined inoculation treatments except treatment T10 (Fig. 3).

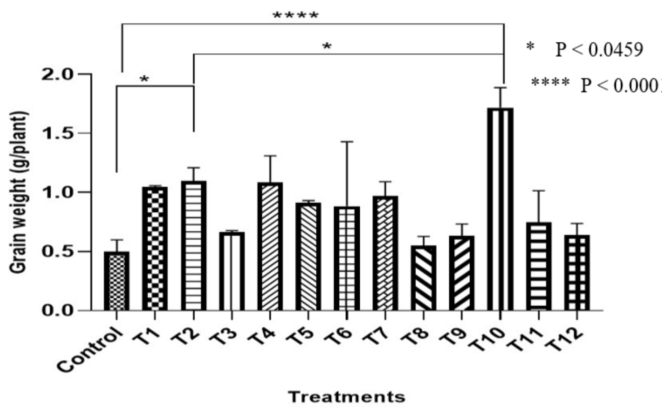

Figure 3. Effect of compatible and incompatible endophytic bacteria on grain weight of chickpea plant.

4. DISCUSSION

The present study showed that, all the six-individual inoculations of bacteria to chickpea seeds confirmed increase in dry shoot, dry root and grain weight as compared to control treatment. This may be due to that all strains inoculated individually were able to promote plant growth as all have capability to produce IAA, solubilise phosphate and siderophore production activity. These findings are similar to the finding of Santiago[12], *et al.* confirming that on individual bacterial inoculation (R170, R168 and R182) significant increase in growth of potato seeding were observed in comparison to control treatment. Molina-Romero[13], *et al.* also confirmed in their study that individual inoculation significantly increases the plant dry weight with respect to uninoculated control. In our study, combined inoculation treatment T9(ME9+BM5+P36) showed the most significant increase in the chickpea plant root and shoot dry weight (600 % and 225 %) and grain weight (250 %) compared to control treatment. It was also found that these cultures showed synergistic compatibility with each other. This may be due to the synergistic action as they did not inhibit growth of each other when applied in the combined form to chickpea seed thus these are beneficial to the plant growth. Similar results were reported by Santiago *et al.*[12] for potato field experiments, where compatible bacterial coinoculation was reported to significantly improve potato growth. Molina-Romero[13], *et al.* also confirmed, in their study, that the use of a compatible bacterial consortium always showed higher value of plant growth parameters as compared to the single inoculated and noninoculated plants. In our study, it was also observed that incompatible bacterial co-inoculation either decreased the growth parameter or resulted in their value similar to those with single inoculation. For example, treatment T8 (HE8+BM5+P36) showed lower shoot dry weight, root dry weight and grain weight than individual inoculation (HE8, BM5 and P36). This may be an outcome of the fact the bacteria act antagonistically in the consortium or inhibited the growth of each other and, thus do not promote the plant growth. This is in agreement with the conclusion drawn by Oliveira[11], *et al.* reported that when the mixture of *Herbaspirillum* (*H. seropedicae* and *H. rubrisubalbicans*) was inoculated to the sugarcane plant, both the cultures were found in the different places in the plant and showed antagonistic behaviour with each other.

5. CONCLUSIONS

On the basis of this study, it can be emphasised that a compatibility test among the various members of a bacterial consortium is necessary prior to its application. This results in the requirement of prior knowledge about the synergy among the different members of the consortium, and can help in predicting the beneficial or disadvantageous effects of the application of a consortium on plant growth.

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