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L-TRYPTOPHAN AMENDMENTS ENHANCED AUXIN PRODUCTION AND GROWTH OF *TRITICUM AESTIVUM* L. BY RHIZOBACTERIA

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ABSTRACT

The objective of the present study was to evaluate the effect of co-inoculations of auxin producing *Bacillus* and *Streptomyces* strains to enhance the growth of *Triticum aestivum* L. For auxin production, *Bacillus cereus* McR-3, *B. subtilis* McR-7, *Enterobacter cloacae* FR, *Streptomyces* sp. RSF, *S. macrosporeus* SCF and *S. griseoincarnatus* CTF were screened by using colorimetric method. Auxin production was determined in the absence and presence of precursor i.e. L-tryptophan (0, 100, 300, 500 µg ml^{-1}). Highest auxin production was recorded with *B. subtilis* McR-7 (31 µg ml^{-1}), *B. cereus* McR-3 (28 µg ml^{-1}) and *E. cloacae* (95 µg ml^{-1}), respectively, in the presence of 100, 500, 500 µg ml^{-1} L-tryptophan. After *in vitro* auxin screening, strains were evaluated for their growth stimulatory effects on *T. aestivum* under axenic conditions. In pot trials, strains showed variable growth responses when inoculated with single or mixed cultures. In single cultures, significant increase for root length (49%), shoot length (42%) and shoot fresh biomass (31%) was recorded respectively with *Streptomyces* sp. RSF and *S. macrosporeus* SCF. For mixed cultures, McR-3+SCF, FR+RSF and McR-7+SCF were the most promising to enhance shoot length (35%), root length (18%) and shoot fresh biomass (20%), respectively, over control. Overall, single and a few combinations of mixed cultures were most suitable to enhance growth of *T. aestivum* under axenic conditions. Results also indicated that consortia comprising of plant growth promoting rhizobacteria (PGPR) and *Streptomyces* offer good potential for the formulations of bio-inoculants for field trials.

Keywords: Bacterial auxin production, L-tryptophan, Rhizobacteria, *Triticum aestivum*, Plant growth promotion

INTRODUCTION

The rhizosphere, the zone of soil under the direct influence of plant roots is a very suitable habitat for the survival and proliferation of rhizobacteria. Root associated rhizobacteria interacts in a beneficial way to influence soil fertility and plant health (Sørensen, 1997). Plants interactions with rhizobacteria are very complex; some of those are beneficial, neutral or detrimental. Globally, there is a growing demand for ecologically and environment friendly approaches in agricultural practices for improving the growth of agricultural crops. Plant growth promoting rhizobacteria (PGPR) is a group of microbes that interact and colonize plant roots and increase their vigor and yield (Wu et al. 2005). Plants produce a wide variety of organic compounds such as phytohormones, carbohydrates, organic acids or vitamins that can be used as nutrients or signals by plant associated microbial communities. PGPR release phytohormones or volatile compounds that mainly act directly to regulate plant growth and morphogenesis (Ryu et al. 2003, 2005).

Plant hormones are signal molecules that act as chemical signals and play a vital role in plant development. These chemical compounds trigger physiological and morphological responses of plant growth

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regulators by rhizobacteria is considered a one of the most significant ways to enhance plant growth (Spaepen et al. 2007). The phytohormones producing potential is also widely distributed among soil and plant associated bacteria. A number of studies have shown that the rhizobacteria can trigger plant growth and yield through the production of auxin; especially, indole-3-acetic acid (Spaepen et al. 2008; Ali et al. 2010). Similarly, gibberellines, cytokinin and ACC-deaminase activity of rhizobacteria also contribute to regulate different developmental aspects of plants (Glick et al. 1998; Timmusk et al. 1999; Bottini et al. 2004). However, currently IAA synthesizing rhizobacteria is well-investigated plant growth regulator. Rhizobacteria synthesize IAA from L-tryptophan by using various pathways; nevertheless, in lower levels, it can be produced L-tryptophan independent pathways (Tsavkelova et al. 2006; Spaepen et al.2007). Bacteria growing within the rhizosphere have the ability to stimulate plant growth by contributing an extra source exogenous microbial IAA. Analysis of plants that were grown in the presence of bacterial inoculations indicated high level of endogenous IAA with significant increase in growth and yield of agronomic plants (Arkhipova et al. 2005; Ali et al. 2010). The release of precursor (L-tryptophan) by plant roots may result in microbial transformation to IAA. IAA produced by bacteria can mediate plant growth only if the secreted IAA is subjected to plant uptake and not metabolized by other soil microbes (Martens and Frankenberger Jr. 1994). Extensive research work has been carried out that indicated the involvement of microbial phytohormones in altering plant growth and development. Plant growth trials conducted by using wild type in comparison with mutant strains (diminished in IAA) demonstrated that the secretion of IAA by *Bacillus amyloliquefaciens* is closely linked with plant growth stimulation (Idris et al. 2007).

In present study, strains used were from genus *Bacillus*, *Enterobacter* and *Streptomyces*. *Bacillus* and *Enterobacter* strains were screened for their *in vitro* auxin production ability to enhance the growth of agronomic plants like wheat (*Triticum aestivum*). *Streptomyces* were used to observe their growth promoting effects when co-inoculated with *Bacillus* or *Enterobacter*. Hence, previously isolated strains such as *Bacillus subtilis* McR-3, *Bacillus Cereus* McR-7 and *Enterobacter cloacae* FR were evaluated as crop inoculants either as single or mixed cultures with strains of *Streptomyces*.

MATERIALS AND METHODS

Bacterial strains

Previously isolated strains of *Bacillus* cereus McR-3, *B. subtilis* McR-7 and *Enterobacter cloacae* FR (Sadiq and Ali, 2013) were used in present study. For co-inoculation experiments, *Streptomyces* sp. RSF, *S. macrosporeus* SCF and *S. griseoincarnatus* CTF were used (Sajid, 2009). Strains were also characterized morphologically and biochemically as mentioned in Cappuccino and Sherman (2002).

Bacterial auxin production

Production of auxin by bacterial strains in liquid cultures was detected colorimetrically. L-tryptophan was amended in 25 ml L-broth medium to a final concentrations of 100, 300 and 500 µg ml^{-I}. Bacterial control was left without L-tryptophan for comparison. After inoculation, bacterial cultures were grown in flasks at 37 ^{o}C for 72 h on shaker. After incubation, cultures were centrifuged at 5000 rpm for 10 min. Finally, 1 ml supernatant was thoroughly mixed with 2 ml of salkowski reagent (Glickmann and Dessaux, 1995). The mixture was kept in dark for 30 min for the development of pink color and optical densities were recorded at 535 nm. Finally, auxin production was calculated from

standard curve that was constructed by using various concentrations of authentic IAA.

Seed sterilization

Seeds of wheat (*Triticum aestivum* L.) were procured from regional office of Punjab Seed Corporation. Seeds of uniform size were sterilized in 0.1% $HgCl_2$ for 3-5 min followed by washings with sterilized distilled water at least three times to remove traces of $HgCl_2$.

Preparation of inoculum

For inoculum preparation, strains were grown overnight in 50 ml L-broth medium at 37^{o}C. After incubation, cells were harvested and suspended in autoclaved distilled water to adjust optical density by spectrophotometer to a final concentration of 10^7 CFU ml^{-1}. Seeds were treated with 20 ml bacterial cultures for 25-30 min. Control seeds were treated with sterilized water for the same duration.

Pot trials

All six strains i.e *Bacillus cereus* McR-3, *B. subtilis* McR-7, *Enterobacter cloacae* FR, *Streptomyces* sp. RSF, *S. macrosporeus* SCF and *S. griseoincarnatus* CTF were used as single culture to inoculate *T. aestivum* under axenic conditions. Similarly, these strains were also used in different combinations; McR-7+RSF, McR-7+SCF, McR-7+CTF, McR-3+RSF, McR-3+SCF, McR-3+CTF, FR+RSF, FR+SCF, FR+CTF. Strains were grown as mentioned above and then mixed in equal quantities to a final volume of 20 ml. After seed bacterization, pots were filled with 110 g autoclaved soil. Three pots were inoculated for each strain or combination in duplicate. Six seeds were sown in each pot. All the pots were arranged in completely randomized design. After seedling emergence, 5 uniform plants were kept in each pot. All pots were incubated at 25^{o}C under photoperiod of 16 h in plant growth chamber. After two weeks, plants were harvested and growth parameters including shoot length, root length, fresh and dry biomass were recorded.

Statistical analysis

Data for bacterial auxin production and growth parameters was subjected to analysis of variance (ANOVA) by using SPSS 16 program (SPSS Inc., Chicago, IL). Means of different treatments were compared by using Duncan's multiple range test (P = 0.05).

RESULTS

Characterization of bacterial strains

Strains belonging to genus *Bacillus* and *Streptomyces* were characterized morphologically and biochemically. For *Bacillus*, overall size of colonies ranges from 2 to 5 mm. Colonies of *Enterobacter cloacae* FR, *B. cereus* McR-3 and *B. subtilis* McR-7 were circular, irregular or rhizoidal, respectively. Strains *B. cereus* McR-3 and *E. cloacae* FR had entire margins while *B. subtilis* McR-7 was filamentous. Similarly, all the strains of *Streptomyces* produced irregular colonies with undefined margins. All the strains of *Bacillus*, *Enterobacter* and *Streptomyces* were catalase positive. Strains of *B. cereus* McR-3 and *B. subtilis* McR-7 were oxidase positive while *E. cloacae* were negative. All the strains of *Bacillus* and *Streptomyces* gave negative results of methyl red test.

Bacterial auxin production

Production of IAA in culture supernatants was detected quantitatively by colorimetric methods (Table 1). In the absence of L-tryptophan, *Bacillus subtilis* McR-7 and *Enterobacter cloacae* FR recorded 11 µg ml^{-1} auxin. On the other hand, *B. cereus* McR-3 showed 9, 10 and 29 fold increase in auxin content in the presence of 100, 300 and 500 µg ml^{-1} L-tryptophan, respectively, over un-supplemented control. Similarly, *B. subtilis* McR-7 recorded 3, 2 and 2 fold increase with increasing precursor concentrations. Whereas *Enterobacter cloacae* FR showed up to 8 fold increase over respective control. *In vitro*

screening of *Streptomyces* strains did not showed any sign of auxin production.

Bacteria-Plant experiments

Bacterial strains were evaluated as single and mixed cultures to evaluate their phyto-stimulatory effects on *Triticum aestivum* L. in pot trials. For single culture inoculations, *Streptomyces macrosporeus* SCF significantly enhanced shoot length by 42% (Fig. 1). Significant increase of 32% and 30%, respectively, were also recorded with *Streptomyces* sp. RSF and *S. griseoincarnatus* CRF.

Table 1. L-tryptophan dependent *in vitro* auxin production by rhizobacteria

Strains	Auxin production (µg ml^{-1})			
	L-tryptophan (µg ml^{-1})			
	0	**100**	**300**	**500**
Bacillus cereus McR-3	01 (a)	10 (b)	11 (a)	28 (b)
B. subtilis McR-7	11 (a)	31 (b)	24 (b)	21 (a)
Enterobacter cloacae FR	12 (a)	68 (c)	93 (c)	95 (c)

Mean of 3 replicates. Different letters within same column in parenthesis indicates significant difference between treatments using Duncan's multiple range test (P = 0.05).

For mixed cultures, McR-3+SCF, FR+SCF, McR-7+RSF, McR-7+SCF showed, respectively, 35%, 35%, 33% and 31% improvements in shoot length over water treated control (Fig. 1). In case of root length, maximum increments of 49%, 44% and 47% recorded with RSF, SCF and CRF, respectively (Fig. 2). On the other hand, mixed cultures inoculations showed statistically comparable results to that of control for root length and number of roots. For number of roots, CTF was statistically significant over other strains and control (Fig. 3) For shoot fresh biomass, SCF, RSF, CRF, McR-7+SCF showed significant enhancements of 32%, 20%, 21% and 20%, respectively, over control (Fig. 4). Figure 5 showed significant improvements in shoot dry biomass for single or mixed culture inoculations.

DISCUSSION

Present study was mainly related with auxin production potential of rhizobacteria and their growth promoting effects on *Triticum aestivum* L. For this study, auxin producing strains of *Bacillus cereus* McR-3, *B. subtilis* McR-7, *Enterobacter cloacae* FR, *Streptomyces* sp. RSF, *S. macrosporeus* SCF and *S. griseoincarnatus* CRF were used as single and mixed cultures to enhance plant growth under laboratory conditions. In present study, auxin production was determined quantitatively by using colorimetric technique. Colorimetric method that used salkowski reagent is a reliable method to quantify different indolic compounds in bacterial culture supernatants (Glickmann and Dessaux, 1995). Overall, *E. cloacae* FR and *B. subtilis* McR-7 showed the highest levels of auxin production in L-tryptophan amended medium.

Bacteria can mediate plant growth either directly through the production of plant hormones or indirectly by the production of antimicrobial metabolites against soil borne phytopathogens. *Streptomyces* has continued to provide a large number and wide variety of new antibiotics. This group of bacteria produced about 75% of commercially and medically significant antibiotics. Moreover, about 60% of the antibiotics isolated from *Streptomyces* are for agricultural use. Gopalakrishnan et al. (2013) reported the plant growth promoting activities of different strains of Streptomyces. Therefore, in present study, mixed cultures of PGPR in combination with *Streptomyces* were used to inoculate *T. aestivum*. Our experiments showed that most of the bacterial treatments enhanced plant growth parameters under laboratory conditions. For instance, *S. macrosporeus* SCF and McR-3+SCF significantly enhanced shoot length up to 42% and 35%, respectively. For root length, *B. subtilis* McR-7 and McR-3+SCF recorded 26% and 17% increases, respectively, over control. Inoculations of plant with IAA

producing rhizobacteria triggered plant growth as well as increased in planta hormones level that suggested growth response was mediated by microbial IAA (Arkhipova et al. 2005; Ali et al. 2010). In one study, plants inoculated with rhizobia in the presence of L-tryptophan gave the highest root dry biomass with significant uptake of NPK compared to control (Etesami *et al.*, 2009). Similarly, PGPR strains from natural plant settings have been reported to harbor beneficial traits that can be applied to enhance the growth and yield of plants (Akhtar and Ali, 2011). A few bacterial strains such as *B. cereus* McR-3 and *Enterobacter cloacae* used in present study are potential human pathogens but they have the ability to produce auxin. It has been reported that pathogenic species to animals and human are mainly transmitted through food chain. Therefore, pathogenic bacteria can contaminate plant surfaces and actively interact and colonize them as an alternate host (Holden et al. 2009).

CONCLUSION

Bacterial strains have the ability to produce maximum auxin levels in the presence of increasing amounts of L-tryptophan. In single cultures, *E. cloacae* FR were the most effective in enhancing growth of *T. aestivum*. However, a few combinations of mixed cultures also gave promising results for vigorous plant growth under laboratory conditions. Hence, bacterial strains in different combinations can be used to inoculate different crop plants.

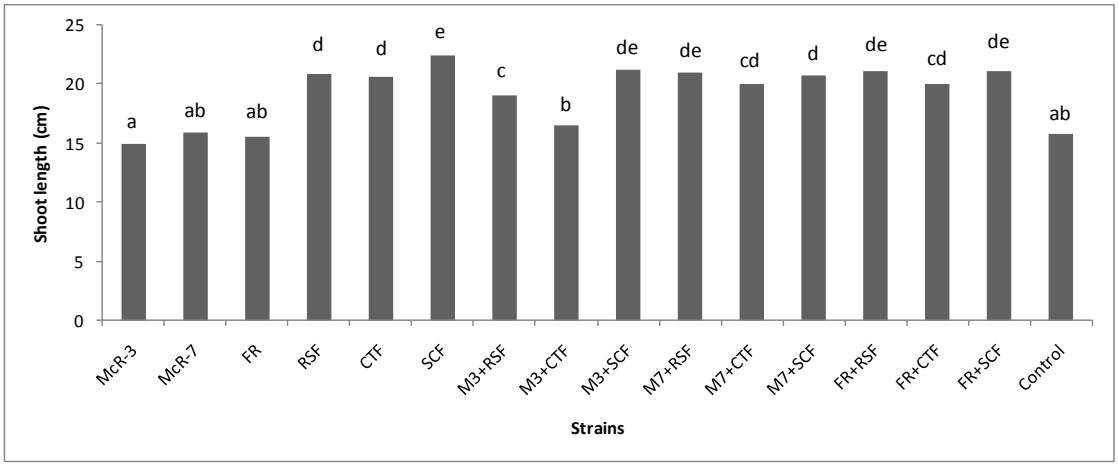

Figure 1. Effect of single and mixed culture inoculations on shoot length of *T. aestivum*. Mean of 30 plants. Different letters on various bars are showing significant difference between treatments for shoot length using Duncan's multiple range test (P = 0.05).

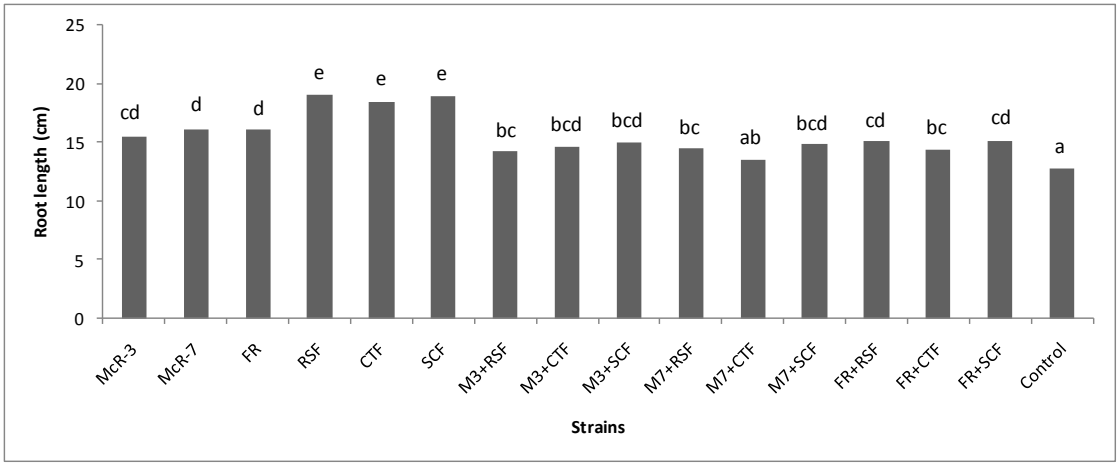

Figure 2. Effect of single and mixed culture inoculations on root length of *T. aestivum*. Mean of 30 plants. Different letters on various bars are showing significant difference between treatments for root length using Duncan's multiple range test (P = 0.05).

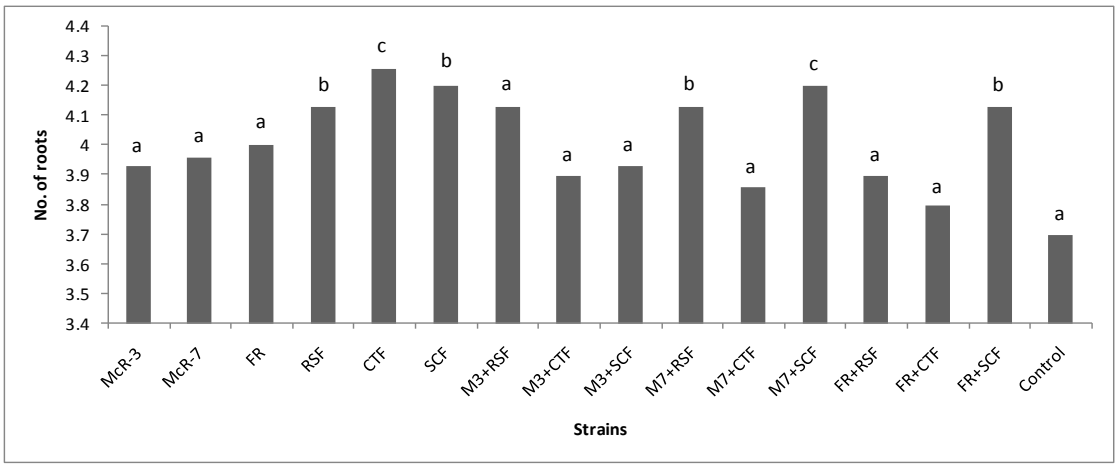

Figure 3. Effect of single and mixed culture inoculations on number of roots of *T. aestivum*. Mean of 30 plants. Different letters on various bars are showing significant difference between treatments for number of roots using Duncan's multiple range test (P = 0.05).

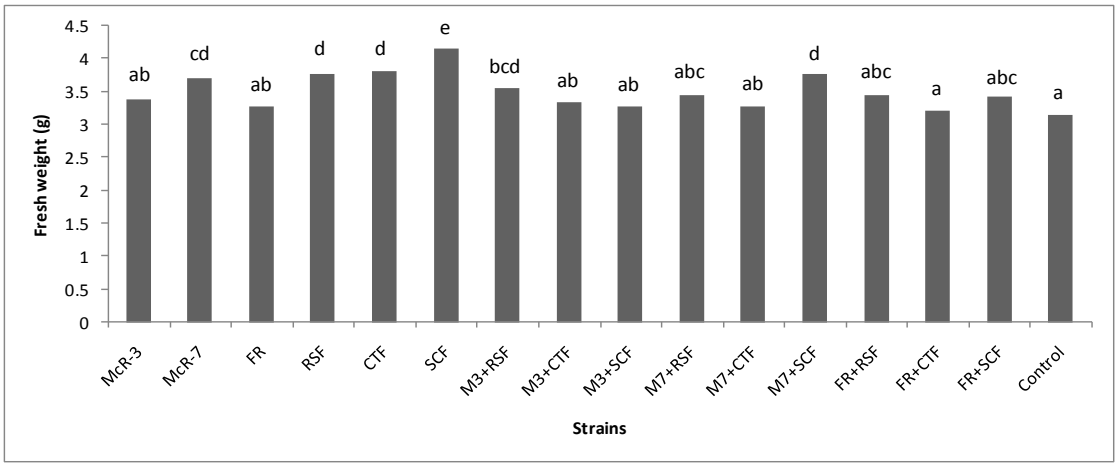

Figure 4. Effect of single and mixed culture inoculations on shoot fresh weight of *T. aestivum*. Mean of 6 plants. Different letters on various bars are showing significant difference between treatments for fresh biomass using Duncan's multiple range test (P = 0.05).

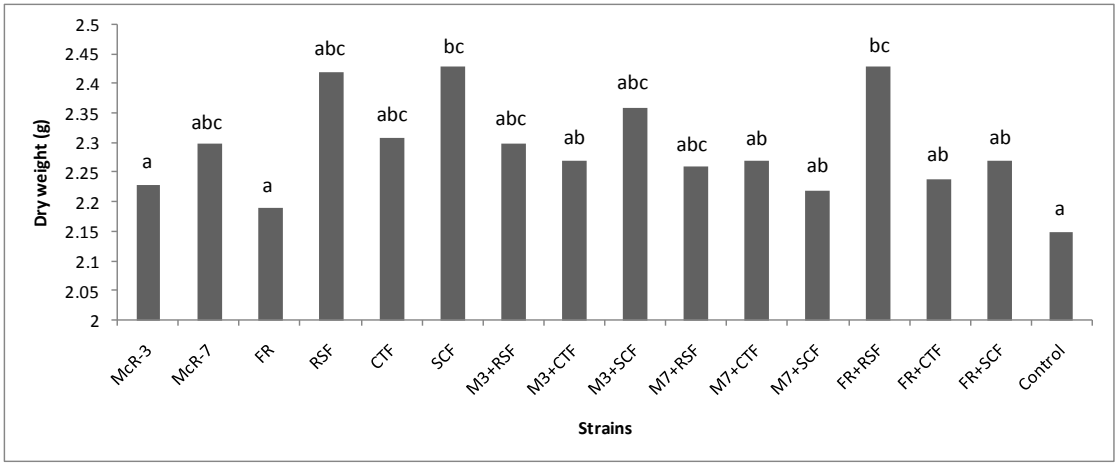

Figure 5. Effect of single and mixed culture inoculations on shoot dry weight of *T. aestivum*. Mean of 6 plants. Different letters on various bars are showing significant difference between treatments for dry biomass using Duncan's multiple range test (P = 0.05).

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