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Effect of PGR producing bacterial strains isolated from vermisources on germination and growth of *Vigna unguiculata* (L.) Walp.

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Abstract

Nineteen bacterial strains were isolated from vermisources and screened for Indole-3-acetic acid (IAA) production among them only nine strains produce IAA and they were identified as *Streptococcus* spp., *Micrococcus* spp., *Klebsiella* spp., *Bacillus* spp., *Enterobacter* spp., *Escherichia* spp., *Alcaligenes* spp., *Erwinia* spp., and *Pseudomonas* spp. Among all other strains *Bacillus* sp. showed the higher IAA production hence selected for further molecular analysis and confirmed as *Bacillus cereus.* The *B. cereus* was grown in nutrient broth supplemented with different concentrations (1, 2, 3, 4 and 5mg/ml) of tryptophan for seven days at pH 7 and at 37°C. Crude IAA was used for in vitro phytostimulatory studies using *Vigna unguiculata* (L.) Walp. The plant growth parameters were analyzed at different day intervals (5, 10 and 15 days). Supplementation of 5 ml crude IAA (2mg/ml of tryptophan) dynamically enhances the plant growth parameters after 15 days.

Keywords: Vermicompost, Tryptophan, Indole-3-acetic acid, *Bacillus cereus, Vigna unguiculata* (L.) Walp

Introduction

To enhance the fertility status of soil, the natural way of feeding the soil with different types of organic inputs has been practiced in recent years. There is an increasing interest in the potential of vermicompost, as plant growth media and as soil amendments. Vermicompost is a product of biodegradation and stabilization of organic materials by interaction of earthworms and microorganisms. It is rich in available nutrients required for plant growth and colonizing microorganisms capable of fertilizing the soil (Karmegam and Daniel, 2000).

Phytostimulatory effects of *Bacillus* strains are well documented and several mechanisms have been suggested for the growth improvement activity of this group of plant growth promoting Rhizobacteria (PGPR). *Bacillus* spp. have several advantages over other PGPR in that they posses several growth promoting traits such as production of phytohormones, secretion of antibiotics, induction of systemic resistance and their use as biopesticides (Reva et al., 2004; Swiecicka et al., 2008). Inoculation of PGPR can elicit physiological changes in plants that are mainly mediated by secondary metabolites especially phytohormones produced by microbes. Bacteria can influence plant growth directly through the production of phytohormones and indirectly through the production of biocontrol agents against soil borne pathogens (Glick et al., 2007). PGPR based products mostly contain strains of *Bacillus* that may have direct agricultural application because of long term viability of these sporogenous bacteria. Therefore, *Bacillus* spp. has the potential to be applied as booster inoculants to increase the efficacy of plant growth promotion and elicitation of systemic disease protection in the field (Kloepper et al., 2004).

Auxins represent a wide group of compounds which are derivatives of the indole ring. Auxins are believed to be essential phytohormones for plant life. Auxins at higher concentrations stimulate the growth of shoots, inhibit that of roots, but concentrations of lower magnitude stimulate root growth. Indole-3-acetic acid (IAA) is the main auxin in plants and it controls many important physiological processes. IAA producing bacteria can potentially interfere with such processes based on the input of IAA into a plants auxin pool (Raja et al., 2008; Sivasankari and Daniel, 2010). This research work was carried out with the main objective to evaluate the IAA production potential of vermisources associated gram-positive bacilli and their phytostimulatory effect on *V. unguiculata* under gnotobiotic conditions.

Materials and Methods

Vermicompost preparation

For the present study epigeic earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny) were collected from the breeding stock of the Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Tamilnadu, India and leaf materials of *Gliricidia sepium* Jacq, *Leucaena*

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lucocephala (Lam.) De Wit and *Cassia auriculata* Linn were collected from Gandhigram campus. The leaf materials were separately subjected to predigestion for 15 days by sprinkling water on heap and covering it with gunny bag and turning it periodically in order to release out the initial heat produced during decomposition of organic materials. The changes in temperature were observed every three days upto 15 days. The vermibeds were prepared in plastic containers and the substrate was moistened to hold 60-80 percent moisture and kept for 24 hours stabilization. 20 numbers of healthy clitellate *E. eugeniae* and 30 numbers of *E. fetida* were separately introduced in different vermibeds per kg of vermibed substrate. Vermicomposting trials were carried out in the rearing room with the relative humidity and the temperature of 75-85 percent and 26-28º C respectively. The substrate was turned (mixed) once in a week and maintained up to 60 days. The experiment was carried out with three replicates for each substrate with proper control (Daniel and Karmegam, 2000).

Microbial study

The total microbial counts in terms of colony forming units (CFU) of bacteria, fungi and actinomycetes in the vermicomposts, vermicasts and earthworm gut (fore gut, mid gut and hind gut) were determined every 15 days (0, 15, 30, 45 and 60 d) using standard plate count method (Parthasarathi and Ranganathan, 1998; Nagarathinam et al., 2000). From the several total colony forming units, only those bacterial colonies which showed predominant growth were selected. The selected 19 dominant bacterial strains were restreaked on to appropriate agar medium to obtain pure cultures and subjected to characterization and identification.

Screening of bacterial strains for production of plant growth regulator Indole-3-acetic acid (IAA)

All the nineteen bacterial strains were separately inoculated in the nutrient broth medium with tryptophan (1 mg/ml) and incubated at 28±2ºC for one week. After a week the cultures were centrifuged at 3000 rpm for 30 min. Two ml of the supernatant was mixed with 2 drops of orthophosphoric acid and 4ml of Salkowski reagent (50 ml of 35% Perchloric acid + 1 ml of 0.5M $FeCl_3$). Development of a pink colour indicates IAA production. Absorbance were measured at 530 nm using Spectronic $20D^+$. The quantity of IAA production was estimated using standard IAA graph and expressed as micrograms per milliliter (Pattern and Glick, 1996; Amaresan et al., 2012). Among the nineteen strains, only nine strains were positive for IAA production. Among all other strains *Bacillus* spp. showed higher production of IAA.

Fermentation process

Different concentrations of L-tryptophan were used to evaluate the in vitro IAA production by *Bacillus* strains in triplicate. For the preparation of inoculum, 50 ml of nutrient broth medium (HiMedia, Mumbai, India) in 250 ml Erlenmeyer flasks supplemented with filter sterilized solution of L-tryptophan in different concentrations such as 0, 1, 2, 3, 4 and 5 mg/ml was inoculated with appropriate overnight bacterial culture and incubated at 28±2ºC for 7 days at 120 rpm **(**OD_{540}=0.45**;** 10^7–10^8 CFU mL^{-1}**).** The cell free supernatant was obtained by centrifuging the fermented broth at 10,000 rpm for 10 min. The supernatant was analyzed for plant growth promotion at various concentrations.

Evaluation of plant growth promoting activity under controlled environment

In vitro phytostimulatory effects of *Bacillus* spp. were demonstrated by supplement the plant with crude IAA at various concentrations i.e. 1, 2, 3, 4 and 5ml at three different day intervals (5, 10 and 15 days) with proper control using *Vigna unguiculata* (L.) Walp. The seeds (30 seeds in each pot) were sown in plastic pots containing sterilized vermiculite and arranged in a completely randomized factorial design. The seedlings were grown in a greenhouse at a temperature of 28–32°C and 85% relative humidity. The pots were watered to 50% water-holding capacity and were maintained at this moisture content by watering to weight every day and plant growth were observed for 15 days. Parameters such as germination percent, shoot length, root length, leaf area, chlorophyll content of the leaves, fresh weight of the whole plant and dry weight of the whole plant were measured using standard procedure.

Statistical Analysis

The following statistical tools were used for the analyses and interpretation of the data. The experimental results are presented in the form of tables and graphs using Microsoft Excel (Version 2003 and 2007). Mean and Standard Deviation were also calculated with the help of the same tool. Data obtained from the different treatments were statistically analyzed using the one-way ANOVA. Differences were considered significant at the 0.05 level using Origin software (Version 8.5.0) 2010, Origin Lab Corporation.

Results and Discussion

Screening of bacterial strains for Indole-3-acetic acid (IAA) Production

The nineteen bacterial strains which showed predominant growth from vermicompost, vermicast and earthworm (fore, mid and hind) guts were screened for IAA production. Nine bacterial strains which showed positive result for IAA production among nineteen strains isolated from vermisources. The observation on the quantity of IAA produced by the nine strains is given in Table 1.

Table 1: Quantity of IAA produced by various bacterial strains isolated from vermisources

S.No	Name of the organism	Quantity of IAA produced (µg/ml)
1	*Streptococcus* spp.	18.78
2	*Micrococcus* spp.	5.99
3	*Klebsiella* spp.	5.69
4	*Bacillus* spp.	18.99
5	*Enterobacter* spp.	6.10
6	*Escherichia* spp.	6.38
7	*Alcaligenes* spp.	10.75
8	*Erwinia* spp.	15.77
9	*Pseudomonas* spp.	18.67

Atiyeh et al., (2002) reported Application of vermicompost increased plant growth, mainly due to production of plant growth regulators by microorganisms during the process of vermicomposting. The dramatic increase in microbial activity in organic matter by earthworms could result in production of

significant quantities of plant growth regulators such as indole acetic acid, gibberellins and cytokinins (Edwards, 1998). Large amounts of humic substances are produced during vermicomposting and these have been reported to have positive effects on plant growth, independent of nutrition. In a nutshell, vermicompost improves physical, chemical and biological properties of soil in the long run on repeated application. The microbial synthesis of plant growth regulators (gibberellin and auxin) is an important factor in soil fertility (Kampert and Strzelczyk, 1975). Indole-3-acetic acid (IAA) and Gibberellin (GA) are secondary metabolites, which are important biotechnological products, produced by many of the microorganisms (Okon and Kapulnik, 1986). Of them, only one strain produced significantly higher rate of IAA and that is *Bacillus* spp. The partial 16S rRNA sequences carried out in the present study for *Bacillus* spp. covered a stretch of approximately 1500 nucleotides for each. About half of the sequences found in the clone library showed only slight relationship to other known sequences, while the other half were highly similar (approximately 95 percent sequence identity) to other database entries for *Bacillus* spp. Less than 0.5 percent of all nucleotides was found to be unique within the conserved regions of the cloned sequence and could almost always be related to reading errors in ambiguous regions of the sequencing gel. The *Bacillus* spp. is identified as *Bacillus cereus*. Beneduzi et al., (2008) had isolated the plant growth promoting strain SVPR30 and identified by 16S rRNA gene sequence as *Bacillus* spp. and they had tested it for plant growth through in vivo experiments. This strain was characterized as a high IAA producer, able to solubilize phosphate and also fix a considerably high amount of nitrogen. The inoculation of rice with *Bacillus* spp. SVPR30 strain showed a significant increase in the root and shoot parts when compared with the controls within 15 and 30 days after sprouting. Numerous *Bacillus* and *Paenibacillus* strains expressed plant growth promoting (PGP) activities and a number of these strains have been commercially developed as generic plant growth promoters. The use of these strains in agriculture has recently been reviewed (Gardener, 2004). Those strains that besides having several PGR properties can also fix nitrogen and solubilize phosphates. Prokryl et al., (1985) have reported about the production of IAA and some other auxins in liquid culture of *Pseudomonas cepacia* and *P. fluoresccens* isolated from maize and bean rhizosphere. Production of growth substances such as auxin (indole-3-acetic acid) by bacteria has also been confirmed in other studies (Frankenberger and Arshad, 1995). Production of growth promoting compound indole-3-acetic acid by *P. polymyxa* has been suggested to be a growth stimulator of crested wheatgrass (Holl et al., 1988).

Evaluation of Plant growth regulating activity of IAA separated from Bacillus cereus (cup study)

In growth study (GS) of *V. unguiculata* the germination percentage of *Vigna unguiculata* seeds sown in vermiculite supplemented with different concentrations of IAA (1, 2, 3, 4 and 5ml) separated from *B. cereus* grown in the medium supplemented with and without (control) different concentrations of tryptophan 1mg/ml (GS1), 2mg/ml (GS2), 3mg/ml (GS3), 4mg/ml (GS4) and 5mg/ml (GS5) and in the control (GS0) is shown in Fig 1. Highest germination percentage (100 percent) was observed in GS2 at 5ml concentration compared to others. This is because when the IAA concentration increases the growth of the plant gets suppressed.

The mechanisms of plant growth stimulation by associative bacteria are most probably related to greater mobilization of nutrients and phytohormone production (Hoflich et al., 1994). They promote seed germination, root elongation and stimulation of leaf expansion (Zimmer, 1995). Lindberg and Granhall, (1985) suggested that greater hormone production is an important part of the overall effect of bacteria on plant growth. They regulate plant growth by modifying physiological and morphological processes at very low concentrations. Observation on growth parameters such as leaf area index, shoot length and root length of *V. unguiculata* grown in vermiculite supplemented with different concentrations of IAA are given in Tables 2 and 3 respectively. The shoot length, root length and leaf area of the plant significantly (P<0.001) increased on 15th day in GS2 at 5ml concentrations of IAA compared to others. When plant growth hormones, such as

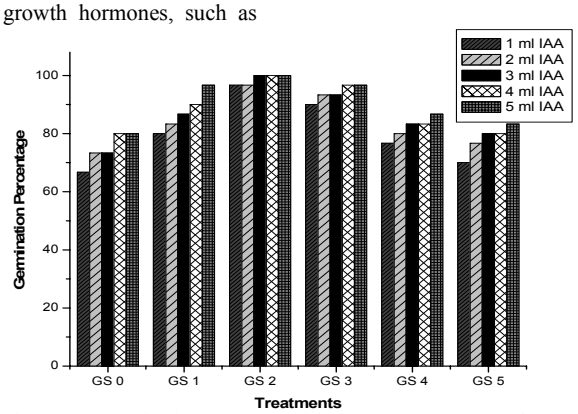

Figure 1: Germination percentage of *Vigna unguiculata* seeds. GS-Growth Study, GS0 - IAA produced by *B. cereus* grown without tryptophan, GS1 - IAA produced by *B. cereus* grown in medium supplemented with 1mg/ml of tryptophan, GS2 - 2mg/ml of tryptophan, GS3 - 3mg/ml of tryptophan, GS4 - 4mg/ml of tryptophan, GS5 - 5mg/ml of tryptophan.

Table 2: Leaf area index of *Vigna unguiculata* grown on vermiculite. GS0 - IAA produced by *B. cereus* grown without tryptophan, GS1 - IAA produced by *B. cereus* grown in medium supplemented with 1mg/ml of tryptophan, GS2 - 2mg/ml of tryptophan, GS3 - 3mg/ml of tryptophan, GS4 - 4mg/ml of tryptophan, GS5 - 5mg/ml of tryptophan at different day intervals (5, 10 and 15d) and in the control (cup study).

Experiment	Quantity of IAA added	Leaf area index(cm^2)		
		5 day	10 day	15 day
GS0 (Control)	1ml	0.38 ± 0.04	1.22 ± 0.06	6.36 ± 0.13
	2ml	0.49 ± 0.05	1.43 ± 0.13	6.96 ± 0.26
	3ml	0.63 ± 0.05	1.74 ± 0.10	7.27 ± 0.27
	4ml	0.68 ± 0.03	1.97 ± 0.19	7.58 ± 0.00
	5ml	0.83 ± 0.03	2.31 ± 0.05	7.83 ± 0.24
GS1	1ml	0.57 ± 0.08	5.07 ± 0.20	9.14 ± 0.19
	2ml	0.68 ± 0.08	5.56 ± 0.12	9.89 ± 0.00
	3ml	0.74 ± 0.12	5.76 ± 0.23	10.21 ± 0.11
	4ml	0.79 ± 0.03	6.64 ± 0.53	11.39 ± 0.12
	5ml	1.03 ± 0.10	6.91 ± 0.26	12.19 ± 0.10
GS2	1ml	1.17 ± 0.07	7.61 ± 0.13	14.53 ± 0.38
	2ml	1.33 ± 0.10	8.48 ± 0.16	15.20 ± 0.39
	3ml	1.37 ± 0.12	8.73 ± 0.29	15.62 ± 0.21
	4ml	1.63 ± 0.05	8.96 ± 0.29	16.74 ± 0.17
	5ml	1.82 ± 0.14	9.84 ± 0.31	17.81 ± 0.43
GS3	1ml	0.73 ± 0.03	5.68 ± 0.20	11.17 ± 0.18
	2ml	0.78 ± 0.03	5.82 ± 0.13	11.57 ± 0.00
	3ml	0.89 ± 0.03	6.37 ± 0.13	12.35 ± 0.19
	4ml	0.99 ± 0.10	7.20 ± 0.23	13.14 ± 0.09
	5ml	1.24 ± 0.12	7.84 ± 0.28	14.17 ± 0.54
GS4	1ml	0.27 ± 0.02	3.79 ± 0.09	8.06 ± 0.17
	2ml	0.35 ± 0.02	4.05 ± 0.11	8.73 ± 0.03
	3ml	0.44 ± 0.07	4.51 ± 0.02	9.41 ± 0.12
	4ml	0.56 ± 0.00	5.10 ± 0.02	10.21 ± 0.33
	5ml	0.65 ± 0.08	5.62 ± 0.11	11.05 ± 0.48
GS5	1ml	0.11 ± 0.00	2.82 ± 0.17	5.83 ± 0.10
	2ml	0.18 ± 0.02	3.26 ± 0.09	6.68 ± 0.16
	3ml	0.20 ± 0.02	3.52 ± 0.01	7.42 ± 0.16
	4ml	0.30 ± 0.02	3.94 ± 0.09	8.11 ± 0.15
	5ml	0.37 ± 0.02	4.46 ± 0.11	8.95 ± 0.15

Table 3: Shoot length and Root length of *Vigna unguiculata* grown on vermiculite. GS0 - IAA produced by *B. cereus* grown without tryptophan, GS1 - IAA produced by *B. cereus* grown in medium supplemented with 1mg/ml of tryptophan, GS2 - 2mg/ml of tryptophan, GS3 - 3mg/ml of tryptophan, GS4 - 4mg/ml of tryptophan, GS5 - 5mg/ml of tryptophan at different day intervals (5, 10 and 15d) and in the control (cup study).

Experiment	Quantity of IAA added	Shoot length (cm)			Root length (cm)		
		5 day	10 day	15 day	5 day	10 day	15 day
GS0 (Control)	1ml	6.10 ± 0.10	6.43 ± 0.12	7.40 ± 0.17	4.13 ± 0.12	4.63 ± 0.12	5.23 ± 0.25
	2ml	6.47 ± 0.06	7.03 ± 0.06	8.13 ± 0.12	4.43 ± 0.06	5.33 ± 0.29	6.00 ± 0.00
	3ml	7.10 ± 0.10	7.60 ± 0.10	8.73 ± 0.12	4.73 ± 0.06	6.17 ± 0.15	6.63 ± 0.12
	4ml	7.73 ± 0.25	8.33 ± 0.29	9.43 ± 0.12	5.37 ± 0.15	6.87 ± 0.15	7.47 ± 0.06
	5ml	8.27 ± 0.25	9.23 ± 0.25	9.83 ± 0.12	6.20 ± 0.20	7.83 ± 0.06	8.30 ± 0.10
GS1	1ml	7.03 ± 0.06	7.47 ± 0.06	8.03 ± 0.06	4.33 ± 0.15	5.30 ± 0.20	6.20 ± 0.10
	2ml	7.24 ± 0.01	8.25 ± 0.01	9.07 ± 0.06	5.33 ± 0.15	6.40 ± 0.26	7.17 ± 0.12
	3ml	8.34 ± 0.01	9.07 ± 0.06	10.17 ± 0.15	6.33 ± 0.15	7.37 ± 0.06	8.40 ± 0.10
	4ml	9.27 ± 0.06	7.31 ± 5.44	11.10 ± 0.10	7.30 ± 0.10	8.47 ± 0.15	9.17 ± 0.06
	5ml	10.20 ± 0.10	11.47 ± 0.12	12.17 ± 0.12	8.43 ± 0.12	9.27 ± 0.15	10.50 ± 0.10
GS2	1ml	10.37 ± 0.06	11.43 ± 0.06	12.17 ± 0.06	9.30 ± 0.10	10.47 ± 0.15	11.27 ± 0.06
	2ml	11.47 ± 0.06	12.57 ± 0.06	13.23 ± 0.06	10.43 ± 0.06	11.37 ± 0.15	12.20 ± 0.10
	3ml	12.40 ± 0.10	13.43 ± 0.06	14.20 ± 0.10	11.33 ± 0.15	12.40 ± 0.26	13.23 ± 0.21
	4ml	13.33 ± 0.15	14.33 ± 0.21	15.27 ± 0.06	12.47 ± 0.25	9.43 ± 6.87	14.33 ± 0.15
	5ml	14.23 ± 0.06	15.53 ± 0.06	16.10 ± 0.10	13.53 ± 0.21	14.43 ± 0.12	15.43 ± 0.21
GS3	1ml	8.33 ± 0.15	9.50 ± 0.10	10.30 ± 0.10	7.30 ± 0.10	8.27 ± 0.21	9.37 ± 0.15
	2ml	9.33 ± 0.15	10.50 ± 0.10	11.37 ± 0.15	8.43 ± 0.21	9.47 ± 0.06	10.20 ± 0.10
	3ml	10.27 ± 0.15	11.40 ± 0.17	12.30 ± 0.10	9.50 ± 0.20	10.43 ± 0.21	11.20 ± 0.10
	4ml	11.33 ± 0.15	12.50 ± 0.10	13.37 ± 0.15	10.43 ± 0.12	11.33 ± 0.15	12.40 ± 0.20
	5ml	12.40 ± 0.10	13.47 ± 0.06	14.37 ± 0.15	11.43 ± 0.12	12.37 ± 0.06	13.27 ± 0.21
GS4	1ml	4.33 ± 0.15	5.27 ± 0.21	6.30 ± 0.10	2.53 ± 0.15	3.37 ± 0.21	4.53 ± 0.06
	2ml	5.57 ± 0.06	6.40±0.26	7.17 ± 0.06	3.37 ± 0.15	4.53 ± 0.06	5.33 ± 0.12
	3ml	6.40 ± 0.10	7.37 ± 0.15	8.20 ± 0.10	4.33 ± 0.15	5.43 ± 0.15	6.23 ± 0.06
	4ml	7.33 ± 0.15	8.27 ± 0.21	9.27 ± 0.15	5.47 ± 0.21	6.47 ± 0.15	7.23 ± 0.06
	5ml	8.33 ± 0.21	9.53 ± 0.15	10.27 ± 0.06	6.43 ± 0.12	7.33 ± 0.15	8.27 ± 0.21
GS5	1ml	2.47 ± 0.25	3.53 ± 0.06	4.17 ± 0.06	0.83 ± 0.29	1.43 ± 0.12	2.20 ± 0.10
	2ml	3.60 ± 0.10	4.37 ± 0.21	5.13 ± 0.06	1.47 ± 0.21	2.37 ± 0.15	3.27 ± 0.15
	3ml	4.33 ± 0.15	5.53 ± 0.15	6.27 ± 0.06	2.57 ± 0.06	3.60 ± 0.10	4.37 ± 0.15
	4ml	5.57 ± 0.06	6.37 ± 0.15	7.33 ± 0.06	3.43 ± 0.21	4.43 ± 0.06	5.40 ± 0.26
	5ml	6.33 ± 0.15	7.20 ± 0.10	8.33 ± 0.15	4.33 ± 0.15	5.57 ± 0.06	6.33 ± 0.15

IAA are applied at higher concentrations (beyond optimum), they could reduce the growth and development of plants. Egamberdieva (2008) isolated 20 bacterial strains from rhizosphere zones. Among the isolates, the representative bacterial strains identified for increasing plant growth were *Bacillus lentus* 10/1, *Bacillus* sp. 41 / 1, and *Cellulomonas* sp.

had increased the root, shoot and dry weight of peas by more than 26 percent compared to the control. Findings of earlier workers who postulated that reported that the *Pseudomonas* and *Azotobacter* spp. cultured in the medium supplemented with 1 and 2 mg/ml of tryptophan enhanced the root elongation of *Sesbania aculeate* and *Vigna radiata*. At a 5 mg/ml tryptophan concentration these microbes, decreased the root elongation in both *S. aculeata* and *V. radiata.* Such an influence caused by both the isolates, indicated that tryptophan supplementation at a 5 mg/ml concentration is toxic and did not promote root elongation (Ahmad et al., 2005). Thus, our study also indicated that the growth of *V. unguiculata* is dose dependant and can be well correlated with the plant growth hormones produced by *B. cereus* supplemented with various concentrations of tryptophan. The mean total chlorophyll content of the leaves on 5d, 10d and 15d are given in Table 4.

The fresh weight and dry weight of the whole plant are given in Table 5. The effects of auxins on plant seedlings are concentration dependent. i.e. low concentration may stimulate growth while high concentrations may be inhibitory. Supplementation of crude IAA significantly increases the both fresh weight and dry weight of whole plant. The results of all the growth parameters were significantly (P<0.001) higher in GS2 i.e. IAA produced by *B. cereus* (in the medium supplemented with 2 mg/ml of tryptophan) at 5ml concentration, on 15 d compared to others.

Production of plant growth regulators by inoculation with plant growth promoter producing rhizobacteria has also been suggested as one of the most possible mechanisms of action affecting plant growth and biomass. Numerous studies have shown improvement in plant growth and development in response to seed or root inoculation with various microbial inoculants capable of producing plant growth regulators (Zahir et al., 2004). Some microorganisms produce auxins in the presence of a suitable precursor such as L-tryptophan. Different plant seedlings respond differently to variable auxin concentrations and type of microorganisms that produce the auxin (Sarwar, 1994).

Conclusions

The effects of auxins on plant seedlings are concentration dependent. i.e. low concentration may stimulate growth while high concentrations may be inhibitory. The results of all the growth parameters were significantly (P<0.001) higher in GS2 i.e. IAA produced by *B. cereus* (in the medium supplemented with 2 mg/ml of tryptophan) at 5ml concentration, on 15 d compared to others. Production of plant growth regulators by inoculation with plant growth promoter producing rhizobacteria has also been suggested as one of the most possible mechanisms of action affecting plant growth and biomass. Numerous studies have shown improvement in plant growth and development in response to seed or root inoculation with various microbial inoculants capable of producing plant growth regulators (Zahir et al., 2004). Some microorganisms produce auxins in the presence of a suitable precursor such as L-tryptophan. Hence these microorganisms were used to fertilize the many agricultural importance crops without any deleterious effects to the soil atmosphere.

Table 4: Total chlorophyll content of *Vigna unguiculata* plant grown on vermiculite. GS0 - IAA produced by *B. cereus* grown without tryptophan, GS1 - IAA produced by *B. cereus* grown in medium supplemented with 1mg/ml of tryptophan, GS2 - 2mg/ml of tryptophan, GS3 - 3mg/ml of tryptophan, GS4 - 4mg/ml of tryptophan, GS5 - 5mg/ml of tryptophan at different day intervals (5, 10 and 15d) and in the control (cup study).

Experiment	Quantity of IAA added	Total Chlorophyll content (mg/g of fresh leaf)		
		5 day	10 day	15 day
GS0 (Control)	1ml	5.33 ± 0.04	7.54 ± 0.25	9.84 ± 0.05
	2ml	5.11 ± 0.42	7.54 ± 0.25	10.02 ± 0.25
	3ml	5.24 ± 0.10	7.71 ± 0.10	10.04 ± 0.15
	4ml	5.24 ± 0.05	7.74 ± 0.15	10.01 ± 0.10
	5ml	5.50 ± 0.22	7.72 ± 0.06	10.15 ± 0.22
GS1	1ml	6.06 ± 0.21	8.35 ± 0.15	10.70 ± 0.07
	2ml	6.52 ± 0.25	8.74 ± 0.25	11.13 ± 0.11
	3ml	6.97 ± 0.15	9.14 ± 0.06	11.46 ± 0.17
	4ml	7.59 ± 0.10	9.52 ± 0.16	12.00 ± 0.06
	5ml	7.80 ± 0.09	9.97 ± 0.12	12.43 ± 0.16
GS2	1ml	6.79 ± 0.14	9.17 ± 0.07	11.72 ± 0.10
	2ml	7.46 ± 0.06	9.63 ± 0.12	12.24 ± 0.07
	3ml	7.92 ± 0.14	10.15 ± 0.24	12.70 ± 0.16
	4ml	8.48 ± 0.25	10.54 ± 0.07	13.17 ± 0.16
	5ml	8.90 ± 0.09	11.04 ± 0.16	13.77 ± 0.20
GS3	1ml	6.23 ± 0.15	8.33 ± 0.07	11.09 ± 0.12
	2ml	6.80 ± 0.14	8.81 ± 0.21	11.56 ± 0.12
	3ml	7.46 ± 0.06	9.16 ± 0.41	12.02 ± 0.16
	4ml	7.83 ± 0.14	9.79 ± 0.16	12.45 ± 0.06
	5ml	8.25 ± 0.23	10.29 ± 0.16	13.04 ± 0.07
GS4	1ml	5.62 ± 0.15	7.76 ± 0.02	9.63 ± 0.12
	2ml	5.94 ± 0.17	8.08 ± 0.07	10.04 ± 0.06
	3ml	6.35 ± 0.05	8.53 ± 0.11	10.61 ± 0.21
	4ml	6.80 ± 0.14	8.98 ± 0.12	11.37 ± 0.78
	5ml	7.42 ± 0.06	9.38 ± 0.15	11.66 ± 0.17
GS5	1ml	5.05 ± 0.05	7.11 ± 0.05	9.30 ± 0.35
	2ml	5.50 ± 0.25	7.49 ± 0.17	9.90 ± 0.21
	3ml	5.83 ± 0.09	7.97 ± 0.05	10.20 ± 0.16
	4ml	6.28 ± 0.24	8.39 ± 0.23	10.56 ± 0.24
	5ml	6.71 ± 0.14	8.77 ± 0.14	11.04 ± 0.24

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Table 5: Fresh weight and dry weight of whole *Vigna unguiculata* plant grown on vermiculite. GS0 - IAA produced by *B. cereus* grown without tryptophan, GS1 - IAA produced by *B. cereus* grown in medium supplemented with 1mg/ml of tryptophan, GS2 - 2mg/ml of tryptophan, GS3 - 3mg/ml of tryptophan, GS4 - 4mg/ml of tryptophan, GS5 - 5mg/ml of tryptophan at different day intervals (5, 10 and 15d) and in the control (cup study).

Germination study	Quantity of IAA added	Fresh weight of the whole plant (g)			Dry weight of the whole plant (g)		
		5 day	10 day	15 day	5 day	10 day	15 day
GS0 (Control)	1ml	0.71 ± 0.01	0.74 ± 0.00	0.77 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
	2ml	0.72 ± 0.00	0.75 ± 0.00	0.79 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
	3ml	0.73 ± 0.00	0.77 ± 0.00	0.81 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
	4ml	0.75 ± 0.01	0.78 ± 0.01	0.83 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
	5ml	0.77 ± 0.01	0.81 ± 0.01	0.85 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.09 ± 0.00
GS1	1ml	0.76 ± 0.00	0.80 ± 0.00	0.83 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
	2ml	0.80 ± 0.00	0.83 ± 0.00	0.86 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.09 ± 0.00
	3ml	0.83 ± 0.00	0.86 ± 0.00	0.89 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
	4ml	0.86 ± 0.00	0.89 ± 0.00	0.92 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
	5ml	0.89 ± 0.00	0.92 ± 0.00	0.95 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
GS2	1ml	1.10 ± 0.00	1.13 ± 0.00	1.16 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.12 ± 0.00
	2ml	1.13 ± 0.00	1.16 ± 0.00	1.19 ± 0.00	0.11 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
	3ml	1.16 ± 0.00	1.19 ± 0.00	1.22 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
	4ml	1.19 ± 0.00	1.22 ± 0.00	1.25 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
	5ml	1.22 ± 0.00	1.25 ± 0.00	1.28 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
GS3	1ml	0.97 ± 0.00	1.00 ± 0.00	1.03 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
	2ml	1.00 ± 0.00	1.03 ± 0.00	1.07 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.00
	3ml	1.03 ± 0.00	1.07 ± 0.00	1.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
	4ml	1.07 ± 0.00	1.10 ± 0.00	1.13 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
	5ml	1.10 ± 0.00	1.13 ± 0.00	1.16 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.12 ± 0.00
GS4	1ml	0.70 ± 0.00	0.73 ± 0.00	0.76 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
	2ml	0.73 ± 0.00	0.76 ± 0.00	0.80 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
	3ml	0.76 ± 0.00	0.80 ± 0.00	0.83 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
	4ml	0.80 ± 0.00	0.83 ± 0.00	0.86 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.09 ± 0.00
	5ml	0.83 ± 0.00	0.86 ± 0.00	0.89 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
GS5	1ml	0.59 ± 0.00	0.62 ± 0.00	0.65 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
	2ml	0.62 ± 0.00	0.65 ± 0.00	0.68 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
	3ml	0.65 ± 0.00	0.68 ± 0.00	0.71 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
	4ml	0.68 ± 0.00	0.71 ± 0.00	0.74 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
	5ml	0.71 ± 0.00	0.74 ± 0.00	0.77 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00

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