RESEARCH PAPER | Agriculture | Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

Phytohormones Production by Plant Growth Promoting Rhizobacterial Isolates In *Gloriosa superba*.L

KEYWORDS: Phytohormone, IAA, GA_3, Gloriosa superba

S. Megala
Research scholar, Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar- 608 002.

R. Elango
Assistant Professor, Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar- 608 002.

ABSTRACT *Phytohormones are plant growth regulators which have stimulatory effects on plant growth. The plant growth promoting rhizobacteria viz., Azotobacter, Bacillus and Pseudomonas isolates obtained in the present study were able to produce IAA and GA_3. The Azotobacter quantity of IAA produced varied from 18.1 to 73.6 µg of 25 ml^{-1} of broth and GA_3 produced varied from 2.43 to 7.10 µg of 25 ml^{-1} of broth. The Bacillus quantity of IAA produced varied from 15.3 to 69.8 µg of 25 ml^{-1} of broth and GA_3 produced varied from 2.34 to 6.80 µg of 25 ml^{-1} of broth. The Pseudomonas quantity of IAA produced varied from 18.7 to 78.6 µg of 25 ml^{-1} of broth and GA_3 produced varied from 2.51 to 7.41 µg of 25 ml^{-1} of broth.*

INTRODUCTION

Plant growth promoting rhizobacteria effects exerted by some plant-beneficial bacteria are due to the bacterial production of plant hormones, such as, indole-3-acetic acid (IAA), cytokinins and gibberellins (Bloemberg and Lugtenberg, 2001; Bottini *et al.*, 2004). IAA was detected in 80 per cent of bacteria isolated from the rhizosphere (Loper and Schroth, 1986).

Growth substances Indole acetic acid (IAA) and gibberellins (GA_3) were isolated from cultures of *Azotobacter chroococcum* which altered growth of stems, leaves and flowers of peas and tomatoes. Further Brown and Burlinghan, (1968) compared effects of treating tomatoes with GA_3 and with cultures of *A. chroococcum* producing small amounts of GA_3 and IAA. These workers supported the hypothesis that *Azotobacter* produced growth factors which were in sufficient quantity in the inoculum added to alter plant development.

The enhancement of plant growth by members of bacilli strains, such as *Bacillus* and *Paenibacillus*, has been well documented (Mc Spadden and Gardener, 2004). They promote plant growth by a number of mechanisms, including the solubilization of phosphorus and production of phytohormones, such as indole acetic acid (IAA) (Choudhary and Johri, 2009; Lal and Tabacchioni, 2009). Phytohormones such as IAA may indirectly improve Phosphorous acquisition by plants by increasing root growth (Marschner *et al.*, 2011).

The PGPR strains of *Pseudomonas* are Known to produce IAA and GA_3 in the rhizosphere of plants and stimulate the crop growth (Kloepper and Scroth, 1978). Rhizosphere colonizing fluorescent *Pseudomonas* significantly increased growth and yield of crops (Suslow and Schroth, 1982).

Gloriosa superba is one of the important medicinal plant which has attained economic significance in recent times. The large scale cultivation of medicinal plants is gaining importance nowadays due to its pharmaceutical value and therefore several agronomic practices are tried to enhance the biomass production and also to increase the biochemical constituents (active principles). Use of microorganisms in the cultivation of field crops is well documented. The phytohormone production by plant growth promoting rhizobacteria like *Azotobacter, Bacillus* and *Pseudomonas* were studied.

MATERIALS AND METHODS

The rhizosphere soil sample were collected in twenty four loaction in five Districts and isolated by the plant growth promoting rhizobacteria like *Azotobacter, Bacillus* and *Pseudomonas*. The isolation code was mentioned.

PRODUCTION OF PHYTOHORMONES BY PLANT GROWTH PROMOTING RHIZOBACTERIAL ISOLATES

The *in vitro* production of phytohormones such as indole acetic acid (IAA) and gibberellic acid (GA_3) by plant growth promoting rhizobacterial isolates were estimated.

Estimation of indole acetic acid (IAA)

A quantity of 100 ml Waksman's broth for *Azotobacter,* Pikovskaya's broth for phosphate solubilizing bacteria (*Bacillus*), and King's B broth for *Pseudomonas* isolates were prepared and sterilized. Freshly prepared, filter sterilized solution of L -tryptophan was added to each flask to a final concentration of 100 mg^{-1}. One ml of culture broth of plant growth promoting rhizobacterial isolates were inoculated to each flask and incubated at 37°C in dark for seven days.

After incubation, the cultures were centrifuged at 6,000 rpm for 5 min to remove the bacterial cells. The supernatant was brought to pH 2.8 with 1 N HCl. Fifteen ml of the acidified supernatant was taken in 100 ml conical flask and to it equal volume of diethyl ether was added and incubated in dark for 4 h. IAA extraction was done at 4°C in a separating funnel using diethyl ether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, 2 ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gorden and Paleg (1957).

To 0.5 ml of the methanol extract, 1.5 ml of distilled water and four ml of Salper's reagent (1.0 ml of 0.5 M $FeCl_3$ in 50 ml of 35 per cent perchloric acid) were added and incubated in dark for one hour. The intensity of pink colour developed was read at 535 nm in a spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity in the culture filtrate was determined and expressed as *µg* 25 ml^{-1} of culture medium.

Estimation of gibberellic acid (GA_3)

The gibberellic acid production by plant growth promoting rhizobacteria was determined by following the method of Borrow *et al.* (1955).

Preparation of reagents

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

Zinc acetate solution
A quantity of 21.9 g of zinc acetate was dissolved in 80 ml of distilled water and one ml of glacial acetic acid was added and the volume was made up to 100 ml with distilled water.

Potassium ferrocyanide solution
A quantity of 10.6 g of potassium ferrocyanide was dissolved in 100 ml of distilled water.

Procedure
A quantity of 100 ml of Waksman's base medium No.77 broth, Pikovskaya's broth and King's B broth were prepared for *Azotobacter,* phosphate solubilizing bacteria and *Pseudomonas* isolates respectively and sterilized. One ml broth of each isolates of *Azotobacter,* Phosphate solubilizing bacteria and *Pseudomonas* and reference strains for each group were added separately in the respective broth and incubated at 37°C for seven days. After seven days of incubation, the culture was centrifuged at 8000 rpm for 10 min to remove the bacterial cells. Fifteen ml of the culture was pipetted out separately into the test tubes and two ml of zinc acetate solution was added.

After two min, two ml of potassium ferrocyanide solution was added and centrifuged at 8000 rpm for 10 min. Five ml of supernatant was added to five ml of 30 per cent hydrochloric acid and the mixture was incubated at 27°C for 75 min. The blank was prepared with five per cent hydrochloric acid. Absorbance was measured at 254 nm in a UV-VIS spectrophotometer. From the standard graph prepared by using gibberellic acid solutions of known quantities, the amount of GA_3 produced by the culture was calculated and expressed as µg 25 ml^{-1} broth.

RESULT AND DISCUSSION

PHYTOHORMONE PRODUCING POTENTIAL OF PGPR ISOLATES

All the plant growth promoting rhizobacterial isolates obtained from the rhizosphere of *Gloriosa superba* were tested for the production of phytohormones such as indole acetic acid and gibberellic acid. However the quantity of phytohormones production varied between genera *viz., Azotobacter*, *Bacillus* and *Pseudomonas* as well as between species of the same genera too

Indole acetic acid (IAA) and gibberellic acid (GA_3) producing potential of Azotobacter isolates obtained from the rhizosphere soil of Gloriosa superb
All the twenty four isolates of *Azotobacter* produced IAA and GA_3 and quantity ranged from 18.1 to 73.6 µg 25 ml^{-1} broth and 2.43 to 7.10 µg 25 ml^{-1} broth respectively (Table - 1).

The isolate GAt- 1 produced the maximum amount (73.6 µg IAA 25 ml^{-1}) followed by GAt- 13 (69.8 µg IAA 25 ml^{-1}). The minimum amount of IAA was produced by GAt- 14 (18.1 µg IAA 25 ml^{-1}). The IAA production by the reference strain of *A .chroococcum* AZB-1 was (72.4 µg 25 ml^{-1}) of culture medium. The *Azotobacter* isolates GAt- 1 produced the highest quantity of (7.10 µg GA_3 25 ml^{-1}) of culture medium followed by the isolate GAt- 13 which produced (6.94 µg GA_3 25 ml^{-1}) of the culture medium. The minimum amount of GA_3 was produced by GAt – 14 (2.43 µg GA_3 25 ml^{-1}).The reference strain of *A.chroococcum* AZB -1 was (6.99 µg GA3 25 ml^{-1}).

Indole acetic acid (IAA) and gibberellic acid (GA_3) producing potential of Bacillus isolates obtained from the rhizosphere soil of Gloriosa superba
All the twenty four isolates of *Bacillus* produced IAA and GA_3 and quantity ranged from 15.3 to 69.8 µg 25 ml^{-1} broth and 2.43 to 6.80 µg 25 ml^{-1} broth respectively (Table - 2).

The isolate GBm-18 produced the maximum amount (69.8 µg IAA 25 ml^{-1}) followed by GBm-9 (67.4 µg IAA 25 ml^{-1}). The minimum amount of IAA was produced by GBm-11 (15.3 µg IAA 25 ml^{-1}). The IAA production by the reference strain of *B. megaterium* PSB-1 was (68.3 µg 25 ml^{-1}) of culture medium. The *Bacillus* isolates GBm- 18 produced the highest quantity of (6.80 µg GA_3 25 ml^{-1}) of culture medium followed by the isolate GBm- 9 which produced (6.54 µg $GA_3$25 ml^{-1}) of the culture medium. The minimum amount of GA_3 was produced by GBm-11 (2.34 µg GA_3 25 ml^{-1}).The reference strain of *B. megaterium* PSB-1 was (6.63 µg GA3 25 ml^{-1}).

Indole acetic acid (IAA) and gibberellic acid (GA_3) producing potential of Pseudomonas isolates obtained from the rhizosphere soil of Gloriosa superba
All the twenty four isolates of *Pseudomonas* produced IAA and GA_3 and quantity ranged from 18.7 to 78.6 µg 25 ml^{-1} broth and 2.51 to 7.41 µg 25 ml^{-1} broth respectively (Table - 3). The isolate GPf-21 produced the maximum amount (78.6 µg IAA 25 ml^{-1}) followed by GPf-19 (76.5 µg IAA 25 ml^{-1}). The minimum amount of IAA was produced by GPf-17 (18.7 µg IAA 25 ml^{-1}). The IAA production by the reference strain of *P. fluorescens* AUPF-1 was (77.2 µg 25 ml^{-1}) of culture medium. The *Pseudomonas* isolates GPf- 21 produced the highest quantity of (7.41 µg GA_3 25 ml^{-1}) of culture medium followed by the isolate GPf- 19 which produced (7.10 µg GA_3 25 ml^{-1}) of the culture medium. The minimum amount of GA_3 was produced by GPf-17 (2.51 µg GA_3 25 ml^{-1}). The reference strain of *P. fluorescens* AUPF-1 was (7.20 µg GA_3 25 ml^{-1}).

CONCLUSION

The IAA and GA_3 producing potential of *Pseudomonas* isolates was more than that of *Azotobacter* and *Bacillus* isolates. The production of IAA and GA_3 by *Azotobacter, Bacillus* and *Pseudomonas* in the rhizosphere soil of *Gloriosa superba.* The PGPR *Viz., A. chroococcum* (GAt-1), *B. megaterium* (GBm-18), *P. fluorescens* (GPf-21). Showed maximum values with regard to production of IAA and GA_3.

ACKNOWLEDGEMENT

We are thanks to University Grants Commission (UGC), New Delhi, for providing financial assistance under Major Research Project for this medicinal plant.

TABLE – 1
Indole acetic acid (IAA) and Gibberellic acid (GA_3) production potential of Azotobacter isolates obtained from the rhizosphere soil of Gloriosa superb

Sl. No	Isolates code	IAA(µg 25 ml^{-1})	GA_3(µg 25 ml^{-1})
1.	GAt -1	73.6	7.10
2.	GAt -2	50.6	5.09
3.	GAt -3	37.5	3.82
4.	GAt -4	65.7	6.56
5.	GAt -5	39.6	4.14
6.	GAt -6	47.4	4.67
7.	GAt -7	42.1	4.32
8.	GAt -8	66.4	6.78
9.	GAt -9	44.6	4.59
10.	GAt -10	54.2	5.52
11.	GAt -11	34.8	3.64
12.	GAt -12	24.2	2.92
13.	GAt -13	69.8	6.94
14.	GAt -14	18.1	2.43
15.	GAt -15	56.5	5.74
16.	GAt -16	63.2	6.52
17.	GAt -17	57.3	5.91
18.	GAt -18	49.5	4.84

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

19.	GAt -19	61.4	6.40
20.	GAt -20	30.3	3.57
21.	GAt -21	60.5	6.29
22.	GAt -22	27.6	3.28
23.	GAt -23	52.7	5.31
24.	GAt -24	58.4	6.10
25.	*AZB -1	72.4	6.99
SED		0.547	0.019
CD(P=0.05)		1.099	0.040

*Reference strain

TABLE - 2
Indole acetic acid (IAA) and Gibberellic acid (GA_3) producing potential of Bacillus isolates obtained from the rhizosphere soil of Gloriosa superb

SI. NO	Isolates code	IAA (µg 25 ml^{-1})	GA_3 (µg 25 ml^{-1})
1.	GBm -1	58.7	5.82
2.	GBm-2	28.7	3.27
3.	GBm-3	49.7	4.92
4.	GBm-4	60.4	5.94
5.	GBm -5	25.4	3.10
6.	GBm-6	44.2	4.42
7.	GBm-7	39.7	4.02
8.	GBm-8	56.4	5.63
9.	GBm -9	67.4	6.54
10.	GBm -10	41.8	4.13
11.	GBm -11	15.3	2.34
12.	GBm-12	50.9	5.12
13.	GBm -13	65.3	6.32
14.	GBm -14	48.5	4.75
15.	GBm -15	34.3	3.67
16.	GBm -16	52.1	5.33
17.	GBm -17	22.7	2.79
18.	GBm -18	69.8	6.80
19.	GBm -19	37.6	3.83
20.	GBm -20	46.3	4.61
21.	GBm-21	62.8	6.10
22.	GBm -22	18.5	2.64
23.	GBm -23	54.3	5.42
24.	GBm -24	30.4	3.41
25.	*PSB - 1	68.3	6.63
SED		0.348	0.029
CD(P = 0.05)		0.699	0.060

*Reference strain

TABLE - 3
Indole acetic acid (IAA) and Gibberellic acid (GA_3) producing potential Pseudomonas isolates obtained from the rhizosphere soil of Gloriosa superba

SI. No	Isolates code	IAA (µg 25 ml^{-1})	GA_3 (µg 25 ml^{-1})
1.	GPf -1	72.9	6.47
2.	GPf -2	23.7	2.57
3.	GPf -3	37.4	3.43
4.	GPf -4	21.6	2.54
5.	GPf -5	16.5	2.50
6.	GPf -6	25.4	2.65
7.	GPf -7	57.9	4.93
8.	GPf -8	68.4	6.14
9.	GPf -9	58.7	5.33
10.	GPf -10	39.5	3.62
11.	GPf -11	45.6	4.00
12.	GPf -12	61.8	5.65
13.	GPf -13	74.8	6.92
14.	GPf -14	42.7	3.87
15.	GPf -15	64.3	5.85
16.	GPf -16	33.2	3.20
17.	GPf -17	18.7	2.51
18.	GPf -18	54.3	4.84
19.	GPf -19	76.5	7.10
20.	GPf -20	48.4	4.32
21.	GPf -21	78.6	7.41
22.	GPf -22	51.6	4.73
23.	GPf -23	66.7	5.91
24.	GPf -24	29.6	2.86
25	*AUPF-1	77.2	7.20
SED		0.298	0.039
CD(P=0.05)		0.599	0.080

*Reference strain

REFERENCE
1. Bloemberg, G.V. and B.J. Lugtenberg. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr. Opin. Plant Biol., 4 : 343-350. | 2. Bottini, R., F. Cassan and P. Picolli. 2004. Gibberelin production by bacteria and its involvement in plant growth promotion and yield increase. Appl. Microbiol. Biotechnol., 65 : 497-503. | 3. Loper, J.E. and M.N. Schroth. 1986. Influence of bacterial source of Indole -3-acetic acid biosynthetic on root elongation of sugar beet, Phytopathol., 76: 386-389. | 4. Brown, M.E. and S.K. Burlingham. 1968. Production of plant growth substances by Azotobacter chroococcum. Jour. Gene. Microbiol., 53 (1) : 135 – 144. | 5. McSpadden Gardener, B.B. 2004. Ecology of Bacillus and Paenibacillus spp. in Agricultural Systems. Phytopathol., 94 : 1252 – 1258. | 6. Choudhary, D.K. and B.N. Johri. 2009. Interactions of Bacillus spp. and plants–With special reference to induced systemic resistance (ISR). Microbiol. Rese., 164 : 493 – 513. | 7. Marschner, P., D. Crowley and Z. Rengel. 2011. Rhizosphere interaction between microorganisms and plants govern iron and phosphorus acquisition along the root axis – model and research methods. Soil Biol. Biochem., 43 : 883 - 894. | 8. Kloepper, J.W., and M.N. Schroth. 1978. Plant growth promoting rhizobacteria on radishes. Proc . 4th Int. Conf. Plant path. Bact., 4: 879 – 882. | 9. Suslow, T.V. and M.N. Schroth. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopath., 72 : 111 – 115. | 10. Gorden, S.A. and L.G. Paleg. 1957. Quantitative measurement of Indole acetic acid. Physiol. Plantarum., 10: 347 - 348. | 11. Borrow, A., P.W. Brain, U.E. Chester, P.J. Curtis, H.G. Hemming, E.C. Jeffereys, R.B. Lloyd, I.S. Nixon, G.L.F. Norris and N. Radley. 1955. Gibberellic acids a metabolic product of the fungus Gibberella fujikuroi some observations on its production and isolation. Jour. Sci. Food. Agric., 6: 340 - 348.