International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(4): 747-757 December 2014
DOI Number: 10.5958/2230-732X.2014.01383.7

GENETICS AND PLANT BREEDING

In vitro evaluation of plant growth regulators on tissue culture bioassay produced by *Pseudomonas* species

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Paper No. 264 Received: 10 July, 2014 Accepted: 15 November, 2014 Published: 18 December, 2014

Abstract

Fluorescent *Pseudomonas* sp. is emerging as largest and potentially most promising group of PGPR (plant growth promoting rhizobacteria) that are involved in plant growth enhancement. Plant growth regulators viz., auxins, cytokinins and gibberellins help in plant growth and development. In present study, fluorescent *Pseudomonas* strains isolated from rhizospheric soil from temperate fruit zone of Himachal Pradesh were investigated for plant growth regulator production i.e. auxins, gibberellins and cytokinins in nutrient broth. All the strains tested produced plant growth regulators in concentrations auxins (1.83-21μg/ml), gibberellins (116.1-485.8μg/ml) and cytokinins (45.4-295.4μg/ml). Two strains (An-1-kul and An-13-kul) were selected on the basis of over all PGPR activities for production of growth regulators. Molecular characterization of best selected *Pseudomonas* strains were done by 16S-rRNA technique. Plant growth regulators produced by best isolates were further studied to observe their effect on growth of callus formation, shoot formation of broccoli and root elongation of cabbage

Highlights

In recent study we evaluated that partially purified cytokinins and auxins extracted from *Pseudomonas aeruginosa* showed maximum callus formation and shoots regeneration in broccoli cotyledon explants *in vitro* developed shoots and development of roots at concentration of 2.0mg/l.

Keywords: Plant growth regulators, auxins, gibberellins, cytokinins, PGPR, Callus formation, root elongation.

Fluorescent Pseudomonads are considered to promote plant growth directly and indirectly and kept under plant growth promoting rhizobacteria (PGPR) group. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (auxins, gibberellins, cytokinins etc.), siderophore, HCN and antibiotics (Arshad and Frankenberger, 1992; Ahad *et al*., 2012). Microorganism isolated from the rhizosphere of temperate fruit growing zone (apple and pear orchards) appear to have greater potential to synthesize and release auxins, gibberellins and cytokinins as secondary metabolites and these are responsible for pronounced effect on plant growth and development. Plant growth regulators are the organic substances that influence physiological processes of plants at very low concentrations when produced endogenously by plants, they are referred to as phytohormones (plant hormones),

where as the term plant growth regulators (PGRs) include many synthetic and naturally occurring compounds. Plant growth regulators as "either natural or synthetic compounds including microbial plant growth regulators that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields or facilitate harvesting" (Nickell, 1982). The production of plant growth regulators induces additional root hair and lateral root formation (Tine *et al.,* 1979). Thereby enhancing the plant's ability to take up nutrients from soil and increasing the yield. Production of different phytohormones like indole-3-acetic acid (IAA), gibberellic acid and cytokinins PGPR can increase root surface and length and promote in this way plant development (Kloepper *et al.,* 2007). Several PGPR and free living rhizobacterial species are reported to produce IAA and gibberellic acid in the rhizospheric soil and thereby play a significant role in increasing the root surface area and number of root tips in many plants (Bhattacharya and Jha, 2012; Salisbury and Ross, 1985). Bacteria like *Azospirillum* and *Pseudomonas* sp. produce cytokinins and gibberellins, in addition to IAA. Cytokinins are a class of phytohormones which are known to promote cell divisions, cell enlargement and tissue expansion in certain plant parts. Gibberellins are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury, 1994). In this paper, production of plant growth regulators and their estimation along with their respective methods, by fluorescent *Pseudomonas* strains isolated from rhizospheric soil has been explored in *in vitro* on broccoli and cabbage for stimulation of callus formation and root formation has been studied.

Materials and Methods

Fluorescent Pseudomonads sp. were isolated from soil samples collected from the normal and replant sites of apple and pear rhizosphere. Total 30 *Pseudomonas* strains were isolated and further identified on the basis of morphological biochemical and physiological tests viz., catalase test, oxidase test, growth at optimum temperature i.e. 4°C, 25°C, 37°C and 41°C, fermentative/oxidative metabolism, gelatin liquification.

Screening of isolates for the production plant growth regulators (PGRS)

Pseudomonas sp. isolated from the rhizosphere soil of pear and apple orchards were screened out for the production of plant growth regulators viz., auxins, gibberellins and cytokinins. Quantitative estimation of auxins was done by colorimetric method (Gordon and Weber, 1951) with slight modifications i.e. 2 to 3 drops of orthophosphoric acid was added to 2 ml supernatant and 4 ml of salper reagent (1 ml of 0.5 M $FeCI_3$ in 50 ml of 30 % $HCIO_4$: prepared fresh). The gibberellins were estimated calorimetrically by the method of (Holbrook *et al.,* 1961). Radish cotyledons expansion bioassay test was employed (Letham, 1971) for estimation of cytokinins like substances the radish seeds (*Raphanus sativus* L. cultivars Japanese white) were germinated in total darkness for 48 h at 28° C.

Production, purification and characterization of plant growth regulators

Auxins, gibberellins and cytokinins produced extra cellularly in nutrient broth by *Pseudomonas* sp. at best optimum conditions i.e. 28° C under shake conditions (90 rpm) after 72 hours. Auxins and gibberellins were extracted from 72 h old cell free culture supernatant with diethyl ether and ethyl acetate respectively. Pooled fractions were evaporated in rotary evaporator at 40° C. Similarly cytokinins were extracted from the supernatant with diethyl ether and extracted with equal volume of n-butanol (Mahadvan and Sridhar, 1986). *Pseudomonas* sp. An-1-kul and An-13-kul were grown in nutrient broth for 72 h. at 28 $\pm$ 2° C under shake conditions (90rpm). Supernatants were prepared by centrifugation of cultures at 10,000 rpm for 20 minutes and were stored at 4° C.

Molecular characterization of fluorescent *Pseudomonas* sp. by 16S rRNA technique

Genomic Deoxyribonucleic acid (DNA) was extracted with DNA isolation kit (Bangalore GeNei), and the

Table.1. Physiological and biochemical characteristics of selected isolates of fluorescent *Pseudomonas* isolates

Isolate	-Characteristics-										Growth (on King's B agar)		
	Tween 80 hydrolysis	Metabolism (oxidative)	Lecith-inate	Denitri-fication	Oxidase	Catalase	Gelatin liquification	*MR/ VP	Citrate utili-zation	Indole	4ºC	25ºC	37ºC	41ºC
An-1-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-2-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-3-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-4-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-5-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-6-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-7-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-8-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-9-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-10-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-11-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-12-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-13-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-14-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-15-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-16-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+
An-17-Kul	-	+	-	+	+	+	+	-	-	-	-	+	+	+

An-18-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
An-19-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Ar-1-kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Ar-2-kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Ar-3-kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Ar-4-kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Pn-1-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Pn-2-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Pn-3-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Pn-4-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Pn-5-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Pr-1-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+
Pr-2-Kul	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	+	+

*MR/VP- Methyl red / Voges Proskauer (+) indicates positivity of test; (-) indicates negativity of test

Table 2. Production of plant growth regulators by isolates of fluorescent *Pseudomonas* species

Isolates	Plant growth regulators (µg /ml)		
	Auxins *	Gibberellins**	Cytokinins ***
An-1-Kul	14.72	485.8	172.7
An-2-Kul	7.27	252.2	90.9
An-3-Kul	6.94	203.5	154.5
An-4-Kul	6.00	196.4	81.8
An-5-Kul	5.83	303.5	90.9
An-6-Kul	5.11	205.8	45.4
An-7-Kul	6.00	316.1	45.4
An-8-Kul	21.00	160.0	140.9
An-9-Kul	8.22	143.5	45.4
An-10-Kul	5.50	165.4	77.2
An-11-Kul	9.27	261.2	90.9
An-12-Kul	16.11	174.8	90.9
An-13-Kul	16.92	233.2	90.9
An-14-Kul	10.22	275.4	113.6
An-15-Kul	5.61	324.1	68.1
An-16-Kul	6.94	292.9	63.6
An-17-Kul	7.61	207.7	63.6
An-18-Kul	5.16	299.6	181.8
An-19-Kul	8.55	212.5	90.9
Ar-1-kul	4.77	267.3	136.3
Ar-2-kul	7.33	232.2	181.8
Ar-3-kul	10.43	361.1	227.2
Ar-4-kul	5.88	299.9	90.9
Pn-1-Kul	12.98	419.2	295.4
Pn-2-Kul	1.83	116.1	186.3
Pn-3-Kul	12.76	419.2	249.9
Pn-4-Kul	3.38	251.5	113.6
Pn-5-Kul	2.71	309.6	45.4
Pr-1-Kul	3.60	180.6	68.1
Pr-2-Kul	3.38	254.7	81.8
$CD_{.05}$	3.19	16.41	3.23

Extracellular Production of plant growth regulators i.e. Auxins*, Gibberellins** and Cytokinins*** expressed in terms of concentration (µg/ml) in 72h old supernatant as calibrated from the standard curve of indole acetic acid(IAA); 10-100(µg/ml) , gibberellic acid (GA_3) ;100-1000(µg/ml),kinetin;100-1000 (µg/ml)

16S rDNA gene was amplified by PCR using the set of primers FP-1(GGTCTGAGAGGATGATCAGT) and RP-1(TTAGCTCCACCTCGCGGC) in MJ Mini BIO-RAD personal thermal cycler-100 (PTC-100). PCR amplification was done as follow: denaturation at 94°C for 1 min, annealing at 55°C and elongation at 72°C for 2 min; with a total of 35 cycles (Widmer *et al.,* 1998; Weisburg, *et al.,* 1991). For DNA sequencing,

eluted amplified DNA products of two selected *Pseudomonas* sp. (An-1-kul, An-13-kul) was first purified followed by sequencing in Bioserve Private limited (Hyderabad, India). Similarity searches of the GenBank database were performed with BLAST.

Tissue culture bioassay for detection of plant growth regulators produced by *Pseudomonas* sp. on plant tissue culture

Effect of partially purified plant growth regulators viz., auxins and cytokinins was studied *in vitro* by plant tissue culture technique (Rashid, R. and Bal, S.S. 2010). In this case MS medium supplement with 100mg/L meso-ionositol, 3% sucrose and 0.8% agar was used as basal medium (Murasshige and Skoog, 1962). Different concentrations of partially purified cytokinins ($1.0mg^{-1}$, $1.5mg^{-1}$ and $2.0mg^{-1}$) and auxins ($0.1mg^{-1}$, $0.15mg^{-1}$ and $0.2mg^{-1}$) from An-1-kul and An-13-kul were used in MS basal medium.

Shoot regeneration from cotyledon explants of broccoli (*Brassica oleracea* var. *capitata*)

Cotyledon explants were excised from 10-12 old days aseptically grown seedlings. The edges of cotyledon explants were cut and their surface was gently tapped with sterile scalpel blade to injure them. These explants were cultured in flasks containing MS full strength basal medium supplemented with different concentrations (i.e. $1mg^{-1}$, $1.5ml^{-1}$, $2mg^{-1}$ of partially purified cytokinins from An-1-kul and An-13-kul at 25 ± 2° C incubation period and 35 $\mu molmeter^{-2}sec^{-1}$photosynthetic photon of lux (PPF) white fluorescence light emitted by Philips bulb. Observations were taken at the interval of every 7 days. Explants of broccoli were evaluated for callus formation; percent shoot regeneration and average number of shoots per explants.

Cabbage (*Brassica oleracea* var. *capitata*) root regeneration in *in vitro* developed shoots

Seeds of cabbage were soaked in water for half an hour and then surface sterilized for one minute in 0.1% $HgCl_2$ solution. After that seeds were rinsed three to four time in sterile distilled water and cultured on germination medium i.e. half strength MS basal medium. Aseptically grown 10-12 days old seedlings of cabbage inoculated on shoot regeneration medium and in vitro regenerated shoots were used as a source of explants for root regeneration.

Result: All isolates were found to be Gram negative rods, non-spore forming, and fluorescent with transparent to translucent and gave greenish, brownish, grayish and yellowish pigmentation on King's B medium plates. These strains were also found to be positive for both catalase and oxidase test. They all were found to be positive for gelatin liquification, oxidative metabolism, denitrification tests and showed growth at 25° C, 37° C, 41° C by plate method but none of the isolates showed growth at 4° C (Table 1).

Production of plant growth regulators viz., auxins, gibberellins and cytokinins

The production of auxins like substances by all the thirty strains of *Pseudomonas* sp. ranges from 1.83 to 21μg/ml. The maximum production of auxins was shown by *Pseudomonas* isolates in the range of (14.72-21.00μg/ml). In supernatants production of gibberellins like substances showed the production in the range of 116.1-485.8μg/ml. The maximum gibberellins production was shown by *Pseudomonas* isolates An-1-kul (485.8μg/ml) followed by Pn-1-kul, Pn-3-kul (419.2μg/ml). All isolates of *Pseudomonas* species were found to produce cytokinins in the range of 45.4-295.4μg/ml. The maximum production of cytokinins shown by *Pseudomonas* isolates Pn-1-kul (295.4μg/ml) followed by Ar-3-kul (227.2 μg/ml) and Pn-3-kul (249.9 μg/ml) (Table 2).

Molecular characterization

Using the universal primer set, DNA fragment of the 16S rDNA gene was amplified by PCR. The PCR amplified 16S rDNA region was sequenced and sequence data were analyzed by BLAST and the nearest match from the GenBank data was reported. DNA sequencing and phylogenetic analysis revealed that strain An-1-kul and An-13-kul was shown to have a 98% similarity with *Pseudomonas*

Table 3. Effect of different concentrations of partial purified cytokinins from An-1-kul on callus formation, %shoot regeneration and average shoots per explants from cotyledons of broccoli (*Brassica oleracea* L. var. *italica*) explant

S. No.	Treatments (column purified cytokinins)	Replications	Callus formation				Callus formation	*% Shooting regeneration	**Shoots per explants
			Pre wt.	Post wt.	Incre. wt.	Conc.			
1.	CK_0 (control)	CK_0 1	0.05	0.15	0.10	261.0	+	-	-
		CK_0 2	0.06	0.19	0.13	341.0	+	-	-
		CK_0 3	0.04	0.15	0.11	281.0	+	-	-
		CK_0 4	0.00	0.00	0.00	0.00	-	-	-
		CK_0 5	0.00	0.00	0.00	0.00	-	-	-
		CK_0 6	0.00	0.00	0.00	0.00	-	-	-
	CD (0.05)					1.26			
2.	CK1(1.0mg/l)	CK_1 (1.0mg/l)-1	0.11	0.18	0.07	180.0	+	-	-
		CK_1 (1.0mg/l)-2	0.098	0.23	0.13	340.0	+	-	-
		CK_1 (1.0mg/l)-3	0.09	0.26	0.17	440.0	+	-	-
		CK_1 (1.0mg/l)-4	0.08	0.19	0.11	280.0	+	-	-
		CK_1 (1.0mg/l)-5	0.12	0.26	0.14	360.0	+	-	-
		CK_1 (1.0mg/l)-6	0.00	0.00	0.00	0.00	-	-	-
	CD (0.05)					1.62			
3.	CK_1 (1.5mg/l	CK_1 (1.5mg/l)-1	0.10	0.20	0.10	260.0	+	-	-
		CK_1 (1.5mg/l)-2	0.10	0.33	0.23	600.0	+	-	-
		CK_1 (1.5mg/l)-3	0.15	0.39	0.24	630.0	+	-	-
		CK_1 (1.5mg/l)-4	0.08	0.21	0.13	340.0	+	-	-
		CK_1 (1.5mg/l)-5	0.08	0.27	0.19	500.0	+	-	-
		CK_1 (1.5mg/l)-6	0.08	0.35	0.27	710.0	+	-	-
	CD (0.05)					1.78			
4.	CK_1 (2.0mg/l	CK_1 (2.0mg/l)-1	0.07	0.24	0.17	440.0	+	¼ x100=25	3/4
		CK_1 (2.0mg/l)-2	0.1	0.15	0.05	120.0	+	2/20 x100=10	6/20
		CK_1 (2.0mg/l)-3	0.10	0.18	0.08	200.0	+	-	-
		CK_1 (2.0mg/l)-4	0.03	0.23	0.20	520.0	+	-	-
		CK_1 (2.0mg/l)-5	0.04	0.09	0.05	120.0	+	-	-
		CK_1 (2.0mg/l)-6	0.03	0.08	0.05	120.0	+	-	-
	CD (0.05)					1.78			

aeruginosa DK2 (Accession number NC_018080.1) and 99 % similarity with *Pseudomonas aeruginosa* PA7 (Accession number NC_009656.1) respectively to the sequences within GenBank.

Accession number

The sequences were submitted to GenBank and accession numbers were assigned for isolate KF682468, KF682469 to An-1-kul and An-13-kul respectively.

Table 4. Effect of different concentrations of partial purified cytokinins from An-13-kul on callus formation, %shoot regeneration and average shoots per explants from cotyledons of broccoli (*Brassica oleracea* L. var. *italica*) explant

S. No.	Treatments (column purified cytokinins)	Replications	Callus formation				Callus formation	% Shooting regeneration	Shoots per explants
			Pre wt.	Post wt.	Incr. wt.	Conc.			
1.	CK_0 (control)	CK_0 1	0.05	0.15	0.10	261.0	+	-	-
		CK_0 2	0.06	0.19	0.13	341.0	+	-	-
		CK_0 3	0.04	0.15	0.11	281.0	+	-	-
		CK_0 4	0.00	0.00	0.00	0.00	-	-	-
		CK_0 5	0.00	0.00	0.00	0.00	-	-	-
		CK_0 6	0.00	0.00	0.00	0.00	-	-	-
	CD (0.05)					1.26			
2.	CK_{13} (1.0mg/l)	CK_{13} (1.0mg/l)-1	0.04	0.19	0.15	390.0	+	-	-
		CK_{13} (1.0mg/l)-2	0.02	0.18	0.16	420.0	+	6/4 x 100 =150	2/4
		CK_{13} (1.0mg/l)-3	0.11	0.23	0.12	300.0	+	-	-
		CK_{13} (1.0mg/l)-4	0.09	0.28	0.19	500.0	+	-	-
		CK_{13} (1.0mg/l)-5	0.07	0.165	0.095	240.0	+	-	-
		CK_{13} (1.0mg/l)-6	0.11	0.25	0.14	360.0	+	-	-
	CD (0.05)					1.779			
3.	CK_{13} (1.5mg/l	CK_{13} (1.5mg/l)-1	0.12	0.30	0.18	470.0	+	-	-
		CK_{13} (1.5mg/l)-2	0.10	0.19	0.09	180.0	+	-	-
		CK_{13} (1.5mg/l)-3	0.04	0.14	0.10	260.0	+	-	-
		CK_{13} (1.5mg/l)-4	0.10	0.30	0.20	520.0	+	-	-
		CK_{13} (1.5mg/l)-5	0.12	0.21	0.09	230.0	+	-	-
		CK_{13} (1.5mg/l)-6	0.11	0.25	0.14	360.0	+	-	-
	CD (0.05)					1.779			
4.	CK_{13} (2.0mg/l)	CK_{13} (2.0mg/l)-1	0.00	0.00	0.00	0.00	-	-	-
		CK_{13} (2.0mg/l)-2	0.00	0.00	0.00	0.00	-	-	-
		CK_{13} (2.0mg/l)-3	0.00	0.00	0.00	0.00	-	-	-
		CK_{13} (2.0mg/l)-4	0.135	0.25	0.12	300.00	-	-	-
		CK_{13} (2.0mg/l)-5	0.00	0.00	0.00	0.00	-	-	-
		CK_{13} (2.0mg/l)-6	0.14	0.29	0.15	390.00	-	-	-
	CD (0.05)					1.026			

Effect of partially purified cytokinins from *Pseudomonas aeruginosa* An-1-kul and An-13-kul on callus formation and shoots regeneration in broccoli (*Brassica oleracea* L. var. italice) cotyledon explants

The aim of the study was to use and to standardize tissue culture based bioassays for (PGPR) plant growth regulators and to study mechanism by which PGPR stimulate plant growth and callus formation. The results showed different concentrations of partially purified cytokinins from *Pseudomonas aeruginosa* An-1-kul and An-13-kul were effective in inducing callus formation and shoot regeneration in cotyledon explants of broccoli. The maximum effect

Table 5. Effect of different concentration of partially purified of auxins from *Pseudomonas aeruginosa* An-1-kul and An-13-kul on percent of root regeneration from *in vitro* developed shoots of cabbage (*Brassica oleracea var.capitata*)

Media	Rooting start after	% root regeneration			Mean
		R1	R2	R3	
RM_0	No rooting	-	-	-	-
RM_1(0.1mg/l)	12 days	100	100	100	100
RM_1(0.15mg/l)	13days	25	25	25	25
RM_1(0.2mg/l)	12 days	100	100	100	100
RM_{13}(0.1mg/l)	10 days	25	25	25	25
RM_{13}(0.15mg/l)	10 days	25	25	25	25
RM_{13}(0.2g/l)	No rooting	-	-	-	-

on callus formation and shoot regeneration was shown by partially purified cytokinin from An-1-kul at 2.0mg/l concentration in MS medium (lacking standard and commercial cytokinin) followed by cytokinin from An-13-kul at 1.0 and 1.5 mg/l concentration) (Table 3 and 4) . Our investigation have shown that partially purified cytokinins at different concentrations from both the strains An-1-kul and An-13-kul when added in MS medium (without commercial cytokinin) induced callus formation and shoot regeneration in some replications.

Effect of partial purified auxins from Pseudomonas aeruginosa An-1-kul and An-13-kul on root regeneration in in vitro developed shoots and development of roots

Root growth promotion is often observed to be the initial or sometimes the sole effect of inoculation with PGPR (Holl *et al.,* 1988; Chenway *et al.,* 1988). This type of bioassay is important for effective screening of putative PGPRs and the type of auxins used to mark influence in the percentage of root formation (number of rooted shoots). The maximum percent of root regeneration (100%) in different concentrations of auxins was recorded in rooting medium RM_1 having 0.1 mg and 0.2mg/l concentration of partially purified auxins from An-1-kul. While only 25% root regeneration was observed in rooting media containing 0.15mg/l and 0.1mg/l concentrations of auxins from An-1-kul and An-13-kul after 13 days of incubation, respectively (Table 5).

Discussion: Available literature showed that it was the first preliminary study in India regarding the comparative effect of the plant growth regulators (cytokinins and auxins) obtained *Pseudomonas* strains. Inoculation of soybean callus with PGPR strain of *P. putida* strain G11-32 showed significantly increased the growth of soybean callus in tissue bioassay in medium without vitamins (Chenway and Nelson, 1991). While strain of *P. putida* G 2-8 decreased the callus biomass. The study showed that the composition of growth medium affected the callus biomass formation. Callus biomass have lowest growth when grown on medium without growth regulators and bacterial inoculation had no effect on callus biomass when grown on NAA (naphthalene acetic acid) and kinetin deficient media. But callus biomass was heightest when media contain growth regulators but no vitamins. Contradictory to these result our studies showed positive results on callus formation and initiation of shoot regeneration from broccoli hypocotyl explants. It may be due to that the partially purified cytokinins form *Pseudomonas aeruginosa* strains An-1-kul and An-13-kul have been used upto maximum concentration of 2.0mg/l.% shoot regeneration of 25% and 10% was observed in different replications i.e. 3 shoots per 4 explant and 6 shoots per 20 explant (3/4 and 6/20 shoots) were observed in two replications of treatment with 2.0mg/l of cytokinins from An-1-kul. Similarly only one replication of treatment with 1.0 mg/l of cytokinins from second strain i.e. An-13-kul showed

150% of shoot regeneration and 2 shoots per 4 explant (2/4 shoots). In comparison to our study appropriate effective concentration of cytokinins might have not been produced by inoculated PGPR *P. putida* strains in the study. This difference may also be due to the potential of strains i.e. strain variation and also due to use of partially purified cytokinins.

Rashid and Bal (2010) reported that direct regeneration was significantly influenced by the genotype, hormone and concentration. They obtained maximum shoot regeneration and number of shoots per explant from hypocotyl on MS medium supplemented with kinetin (0.5 mg^{-1}) and BAP (0.5 mg^{-1}). Madhulatha *et al.,* (2006) reported that out of the various combinations of cytokinins and auxins tried, MS medium containing 2.5 mg^{-1} BAP + 0.5 mg^{-1} IAA was the best combination for multiple shoot induction. The direct optimization of shoot regeneration protocol from hypocotyl explant for cotton using thidiazurm (TDZ and 0.08mg^{-1}), Naphthalene acetic acid (NAA 0.01mg^{-1}) and silver nitrate (5.1mg^{-1}) was done (Ouma *et al.,* 2004). It was reported that auxins exerts primary role in root formation by its involvement in successive and interdependent phase (Bellamine *et al.,* 1998). Some finding showed that differentiation of phloem ray parenchyma cells into root primordia depends upon the type and concentration of auxins (Sabatini *et al.,* 1999). Further it has been reported that differentiating cells require the most appropriate auxins to become competent to respond to the organogenic signal by (Blakesley and Chaldecott, 1997). The effect of auxins on rooting was found promotory at low concentration and inhibition at supra-optimal concentration (Sugiyama, 1999). IBA (indole butyric acid) and naphthalene acetic acid (NAA) hormone tested for maximum percentage of rooted shoots, root number, root length and quality of roots (Chanway *et al.,* 1989). Successful rooting was achieved by placing the shoots onto MS basal medium supplemented with 0.20 mg^{-1} NAA (Sharma and Srivastava, 2014). IBA at 1.5mg^{-1} showed maximum root initiation (86.67% shoots), 5.03 numbers of roots per rooted explant and 4, 95 cm root length. Earlier it has also been reported 90-95percent survival rate of root regeneration in plant (Ali *et al.,* 2009). The highest percentage of hypocotyl explants of soy bean producing shoot (96.67%) and the highest mean number of shoots produced per hypocotyl explant (6.03) were obtained on 3 mgl^{-1}BAP. While the highest percentage of shoot tip explants producing shoot (100%) and highest number of shoot produced per shoot tip explant (3.76) were recorded on5 mgl^{-1} BAP. It has been reported that use of BAP (6- benzyl amino purine) at 3 mg^{-1} and 5 mg^{-1} as the most suitable concentration for the callus formation and shoot initiation (Ravanfar *et al.,* 2009). El-Barkry (2002) observed maximum number of shoots on MS basal medium supplemented with 2.0mg^{-1} BA and 0.20 mg^{-1} IAA in five cultivars from cotyledon explants.

From this experimental study, it can be concluded that a tissue culture based bioassay is important and valuable for evaluation of putative PGPRs and for their products like plant growth regulators and further detailed work is necessary to define the conditions under which PGPR activity and their products can be detected. Further experimentation should be under taken to determine if its PGPR activities is related to PGRs production *in situ*. The fluorescent *Pseudomonas* sp. are plant beneficial rhizobacteria with all the direct and indirect plant growth promoting activities like plant growth regulators such as auxins, cytokinins and gibberellins. Plant growth regulator production is an important secondary metabolite of fluorescent *Pseudomonas* as it directly involves in the growth and development of plant root, shoots. It is worth to note that strains belonging to *Pseudomonas aeruginosa* are considered safe for use in biotechnological applications.

Acknowledgements

The authors are highly thankful to the department of Basic Science (Microbiology) and Biotechnology of Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)-173230, India to carry out the present work.

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