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In vitro bioassay of secondary metabolites of soybean (*Glycine max)* plant roots and their effect on growth of bacteria, hormones and plants

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ABSTRACT

Legume plants produce a high diversity of natural secondary metabolites with a prominent function that is important for the communication of the plants with other organisms and are significant for growth and development processes. In the present experiment, 50 roots of soybean [*Glycine max* (L.) Merr], were selected from the fields during 2016 to study the effect of root metabolites on bacterial growth and their growth hormone production potential. Bioassay was performed on the germination of chickea (*Cicer arietinum* L.) plant. The results indicated that the secondary metabolites of legume root enhance bacterial growth. It was found that the bacterial (*Pseudomonas)* growth was concentration dependent and was highest at the highest concentration of root extract, reflected by its maximum cell count. The highest cfu count (254 × 10^5 cfu/ml) of *Pseudomonas* was obtained in culture medium containing 100% root extract after 48 hr incubation. The amounts of IAA and GA produced at this concentration were 387.9 µg/25ml and 103.87 µg/25 ml, respectively. The production of IAA and GA was maximum in root extract containing media. It was observed that root extract was most effective in inducing seed germination and multiple root production. It was concluded that root extract played a vital role in the *in vitro* plant growth hormone (IAA and GA) production and enhancement of growth of chickpea plant.

Key words: Chickpea, GA, IAA, *Pseudomonas,* Root extract, Soybean

The legume family is the third largest plant family next to Orchidaceae and Asteraceae. Legume plants have a significant agricultural importance because they are a crucial source of proteins, vegetable oil and improvement in soil fertility (Sugiyama and Yazaki, 2012). The legume plants produce specific signaling molecules from roots that create biological interests such as symbiotic nitrogen fixation, as well as become important to soil, human and environmental health. The legume plant roots exudates contain an enormous range of potentially valuable small molecular weight compounds which are released into the rhizosphere. Some of the most complex chemical, physical and biological interactions experienced by terrestrial plants are those that occur between roots and their surrounding environment of soil (Bais *et al.* 2006).

Legume plants produce a high diversity of natural secondary metabolites with a prominent function that is important for the communication of the plants with other organisms and are insignificant for growth and developmental processes (Mazid *et al.* 2011). Growth hormones producing rhizobacteria are considered not only to promote plant growth directly or indirectly, but also exhibit a variety of characteristics responsible for influencing plant growth (Shahab *et al*. 2009). Bacteria that colonize the rhizosphere and plant roots and enhance plant growth by any mechanism are referred to as plant growth promoting rhizobacteria (PGPR). Indole-3-acetic acid (IAA) is one of the physiologically active growth hormones that influence plant processes, such as initiation of cell division and promotes vascular differentiation (Thuler *et al.* 2003). With this background, we conducted an experiment to summarize the effect of legume root metabolites on plant growth, rhizosphere microbes and their interactions. We tried to understand how root metabolites enhance *in vitro* growth hormones production through rhizobacteria and importance of root metabolites on the plant vigour index.

MATERIALS AND METHODS

Extraction of root metabolites

In the present experiment, 50 roots of *Glycine max* (L.) Merr. were selected to study the effect of root metabolites on bacterial growth. For this purpose healthy roots of this legume plant were collected from agriculture land of Bhopal, India during *Kharif* season 2016, and washed and sterilized by chemicals. After washing the roots, extraction was done with distilled water by maceration technique. After extraction, crude was concentrated by evaporation.

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In this experiment, *Pseudomonas* was selected as bacterial culture to study the effect of root metabolites on its growth pattern. Four different concentrations (25, 50, 75 and 100%) of root extracts were prepared in distilled water and added in culture medium containing *Pseudomonas*. A culture medium without root extract was used as a control. All cultures were incubated at 37ºC for 48 hrs. After 48 hrs of incubation, cfu count was done. Later all cultures were transferred on a rotary shaker for 7 days of fermentation.

Effect of root metabolites on plant growth hormone production was performed by using quantitative estimation of indole acetic acid (IAA) and gibberellic acid (GA) following the methods of Gorden and Paleg (1957) and Paleg (1965), respectively. After incubation, the cultures were centrifuged at 6000 rpm to remove the bacterial cells, and the supernatant was collected in a conical flask. For the estimation of IAA, pH was adjusted to 2.8 using 1 N HCl in a 100 ml conical flask and 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. The dried material was dissolve in methanol and treated with Sapler's reagent and incubated in dark for 1 hour. After incubation, intensity of developed pink color was read at 535 nm in a spectrophotometer.

To estimate GA, supernatant was taken and treated with zinc acetate the 2 ml of potassium ferrocyanide added and centrifuged at 1000 rpm for 15 min. Five ml of this supernatant, was treated with 5 ml of 30% HCl and incubated at 20°C for 75 min. The absorbance of the sample as well as blank was measured at 254 nm in the spectrophotometer.

To study the effect of root extract on germination rate, the seeds of chickpea (*Cicer arietinum* L.) were prepared for each treatment. For sterilization, seeds were soaked in 2% sodium hypochlorite for 3 min and then they were washed with sterile distilled water 5 times. Sterilized seeds were incubated in 50 ml root extract. The soaked seeds were sowed in a plastic bag filled with equal amount of soil. Treatment with distilled water was used as control and followed the same procudure. On 8th day, the chickpea plants were harvested separately according to the treatment. The effects of different treatments were observed and observation on important plant indices such as root length, shoot height and the number of adventitious roots were taken.

RESULTS AND DISCUSSION

Effect of root secondary metabolites on bacterial growth

Highest cfu count (254 × 10^5 cfu/ml) of *Pseudomonas* was obtained in culture medium containing 100% root extract after 48 hrs incubation Fig 1. Whereas, at the concentration of 25, 50 and 75% root extract showed the bacterial counts of 85 × 10^5 cfu/ml, 117 × 10^5 cfu/ml and 145 × 10^5 cfu/ml, respectively. However, control treatment (without root extract culture medium) had bacterial count of 65 × 10^5 cfu/ml. These observations indicated that the secondary metabolites of legume root enhanced the bacterial growth. It was found that bacterial growth is concentration dependent, as highest concentration gave maximum cell count. Plant roots support the growth of a variety of soil microorganisms that have beneficial or detrimental effects on plant growth (Hynes *et al.* 2008). Previous studies reported that roots release large quantities of metabolites from living root hairs or fibrous root systems. These metabolites act as chemical signals for motile bacteria to move to the root surface, and are the main nutrient sources available to support growth and persistence in the rhizosphere (Nihorimbere *et al.* 2011). In this study, we could find nutritional value of root extract for bacterial growth. Different degrees of growth enhancement of the bacteria by the root extract suggested that the organism was required in good number with secondary metabolites as co-factors (Ghosh *et al*. 2015).

Effect of root extract on the production of plant growth hormones

Root extract of leguminous plant generally induces plant growth hormone production *in vivo,* but present study revealed that the root extract plays great role in the *in vitro* plant hormone production, when comparison was done between extract free and extract containing media (Fig 2). *Pseudomonas* culture containing the root extract was the most efficient in the production of IAA and GA. The amount of IAA and GA produced was 387.9 µg/25 ml and 103.87 µg/ 25 ml, respectively. The production of IAA and GA was maximum in the root extract containing media. These findings show that root extract induced more IAA and GA production by *Pseudomonas* as compared to blank (control). The control produced IAA in the quantity of 91.46 µg/25 ml. GA production was not recorded in control.

Microorganisms release phytohormones, small molecules or volatile compounds, which may directly or indirectly activate plant immunity or regulate plant growth by utilizing plants' organic compounds including sugars, organic acids and vitamins, which can be used as nutrients or signals by microbial populations (Ortz-Castro *et al*. 2009). Plant hormones are a class of organic substances which are synthesized during the plant metabolism and obtained through plant extraction, chemical synthesis as well as microbial fermentation (Shi *et al.* 2017). Other studies suggested that the IAA and GA acte to produce bacteria as efficient biofertilizer inoculants to promote plant growth (Nghia *et al.* 2017). Data obtained from seed germination experiments demonstrated extract specific positive effect on seed germination. The significance of the study could be emence as the potential of these root extract will flourish the plant growth and ultimately IAA and GA production in the field and prevent environmental pollution caused by application of chemical fertilizers.

It was observed that, legume root extract was found to be the most effective in inducing seed germination. All essential nutrients and enzymes which induced microbes for the production of plant growth hormones were present in the root extract. The root extract showed best effects on growth of chickpea. Extract treated plants showed a tremendous effect on root and shoot growth after 8 days

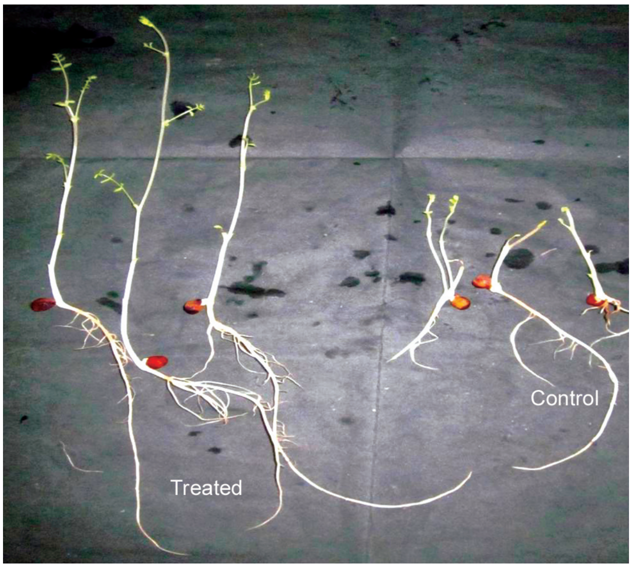

Fig 1 Effect of root extract on shoot and root length of chickpea

Fig 2 Effect of root extract on multiple root production in chickpea.

Table 1 Effect of root extract on shoot and root length of chickpea

Sample ID	Shoot length (in cm)	Root length (in cm)
Control plant	8.4 ± 7	3.4 ± 21
Root extract treated plant	12.5 ± 28	9.96 ± 14

(Table 1 and Fig 1). The highest root length and shoot height observed were 9.96±14 cm and 12.5±28 cm after keeping for 8 days in treated plants. Smallest shoot and root elongation effect was observed in control plants. The shoot and root length of control plant was 8.4±0.7 and 3.4±0.21 cm. Root extract not only induced plant growth but also produced multiple roots in chickpea plant (Fig 2). It induced root hairs production for higher absorption of nutrition from the soil. Yasumoto *et al.* (2011) reported the effects of plant residue, root exudate and juvenile plants of rapeseed (*Brassica napus* L.) on the germination, growth, yield, and quality of subsequent crops in successive and rotational cropping systems and found allelopathic effects of rapeseed plants on the growth, yield, and quality of the subsequent sunflower plant in the same field.

It was concluded that legume root extract has an important role in plant germination and its growth. Besides, it has great ability to enhance bacterial and plant growth. Root extract played a vital role in the *in vitro* plant growth hormone (IAA and GA) production. These results established the beneficial effects of microbial inoculation which may be used for industrial production of these plant growth hormones by using root extract as nutritional substrate.

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