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Optimization Studies on Gibberellic Acid Production by Fungal Strains Isolated from Chilli Rhizosphere

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Abstract
A total of 10 fungal strains were isolated from the chilli rhizospheric soils collected from in and around the Guntur, Andhra Pradesh, India. All the ten strains were observed for the gibberellic acid production. Among them the strain DS5 showed maximum gibberellic acid production of 45.8 µg/ml. environmental factors like incubation period, pH and temperature were observed for gibberellic acid production. Maximum production was recorded at 96 h of incubation and pH 8.0 at 40^{o}C. Carbon and nitrogen sources greatly influenced the gibberellic acid production. Among them sucrose and yeast extract influenced the maximum gibberellic acid production. Results reveals that the fungal strain DS5 was proved to exhibit the best plant growth promoting characterises and it was suggesting for sustainable agriculture.

Keywords
Plant growth promoters (PGP), Gibberellic acid (GA3), Potato dextrose agar (PDA).

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1. INTRODUCTION
Many microorganisms produce the plant growth promoting substances among them Auxins and gibberellins plays a major role in the growth of the plant. Plant growth promoting substances are Indole Acetic Acid, gibberellic acid, siderophores and HCN. Among these substances Gibberellic acids, also known as gibberellins, are the complex organic molecules acting as plant growth hormones. Gibberellins are chemically, diterpenoid acids of which having molecular formula $C_{19}H_{22}O_6$. Several microorganisms such as bacteria and fungi, gibberellic acid 3 is the principal product of gibberellins, and acts as secondary metabolite [Bruckner and Blecschmidt, 1991]. Till now, 136 gibberellins were isolated from various plants, and among that gibberellic acid shows maximum biological activity. The use of GA_3has been approved by Food and Drug Administration (FDA) because of its tremendous application and nontoxic properties, and its safety for environment and human was confirmed by Material Safety Data Sheet (MSDS) [Rodrigues *et al*., 2011]. Majority of the fertilizers are associated with environmental pollution, plant growth hormones like gibberellic acid 3 have to be produced cost-effectively in huge amounts in order to enhance the quantity of agricultural products [Bilkay *et al*., 2010].

In the present study chilli rhizospheric soils were selected for growth hormones production. Chilli is very important crop in the areas of Guntur and Prakasam districts in Andhra Pradesh. Chillies from

Andhra Pradesh are well known for their pungency and good red colour. Many PGP (Plant growth promoting) microorganisms associated in chilli rhizosphere. There is an emergence to know and optimize the important plant growth promoters from chilli rhizosphere. A few reports related to fungi isolated from chilli rhizosphere. The present study was mainly focussed on plant growth promoting Gibberellic acid production by fungal strains isolated from chilli rhizosphere. These strains were used as bio inoculants in sustainable agriculture.

2. MATERIALS AND METHODS

i). Isolation of fungi

The rhizosphere soil samples from the chilli fields of 10 different areas of Guntur, district of Andhra Pradesh, India were collected for the study. Fungal strains were isolated on Potato Dextrose Agar (PDA) medium by soil dilution plate technique (Andres, *et al*., 2003) using 10^{-3} to 10^{-5} dilutions. The plates were incubated at 28 ± 2°C for 5 days. Fungal colonies appeared in the plates were noted and subcultured. After purified by single spore isolation method and they were maintained on potato dextrose agar (PDA) slants. Identification of Fungal solates was based on culture characters as well as microscopic parameters (conidiophores branching, phialides shape and position, spore size and shape) (Nagamani *et al.,* 2006). The pure cultures were stored in the refrigerator at 4°C for further studies.

2. Gibberellic acid extraction and determination

Culture media were filtered, and then samples were acidified to pH 2.5 with HCl and extracted using liquid-liquid (Ethyl acetate/NaHCO3) extraction (Cho *et al*., 1979). Gibberellic acid in the ethyl acetate phase was measured by UV spectrophotometer (Jenway 6105 UV/VIS) at 254 nm (Bruckner B, Blechschmidt, 1991). The amount of gibberellic acid was calculated from the standard curve.

3. Optimization of Physico-Chemical parameters for Gibberellic Acid Production

i). Effect of incubation time on Gibberellic acid production

To determine the optimal incubation time for GA3 synthesis, DS5 was inoculated into nutrient broth incubated for 24, 48, 72, 96, and 120 hours at 30^{o} C on a rotary shaker (200 rpm). After incubation, fungal growth and the GA3 amount were estimated by using spectrophotometer.

ii). Effect of pH and temperature on Gibberellic acid production

Different pH (5.0, 6.0, 7.0, 8.0 and 9.0) were introduced into the nutrient broth DS5 was inoculated for 72 hours at room temperature on a rotary shaker (200 rpm). After incubation, fungal growth and the amount of Gibberellic acid were estimated by using spectrophotometer.

iv). Effect of sugars on Gibberellic Acid Production

To study the gibberellic acid production various (1%) sugars (Arabinose, Sucrose, Maltose, Glucose, lactose and fructose) were supplemented into the nutrient broth medium. After inoculation of DS5 for 72 hours of incubation, fungal growth and the amount of Gibberellic acid were estimated by using spectrophotometer.

v). Effect of nitrogen sources on Gibberellic Acid Production

To study the effect of gibberellic acid production in various (0.5%) nitrogen sources (Peptone, Ammonium sulphate, Beef extract, yeast extract, potassium chloride and L-Aspargine) were supplemented into the nutrient broth medium. After inoculation of DS5 for 72 hours of incubation, fungal growth and the amount of Gibberellic acid were estimated by using spectrophotometer.

3. RESULTS AND DISCUSSION

From the results all the 10 fungal strains showed Indole acetic acid and gibberellic acid production. In the present study we focus gibberellic acid production by 10 fungal strains. Among them DS5 strain showed maximum amount of gibberellic acid production. A few fungal species were reported to produce gibberellic acid. Similarly, Bruckner *et al*., (1989) observed that the Gibberellins as a plant growth regulator is a classic example of the interactions between soil micro-organisms and plants. It is well known that gibberellins were first obtained from culture filtrates of the soil fungus *Gibberella fujikuroi* (Saw.) Wr. (*Fusarium moniliforme*Sheld). Gibberellic acid production was initial at 24 h of incubation and it was reached maximum at 96 h. further increase in incubation period gibberellic acid production was decreased (figure 1). Incubation period was observed for GA_3 production by Fusarium monilifome (Rangaswamy, 2012). 9 days was optimal time for GA_3 secretion by Fusarium fujikuroi SG2 (Udhandi *et al*., 2010). and Fusarium monilifome (Kobomoje *et al*., 2013).

The studies on pH play a major role in the fungal growth and secondary metabolite production. Alkaline and neutral pH was proved to gibberellic acid production. The present strain DS5 showed

maximum production at pH 8.0 it indicates the alkaline pH (Figure-2). Temperature also play a major role for the production of gibberellic acid and in the present strain DS5 showed maximum at 40^{o} C (Figure-3).

Bilkay *et al*., (2010) reported pH 5.0 as optimal time for GA production by Aspergillus niger, whereas pH 7.0 was optimum for GA production by Fusarium monilifome [Rangaswamy V ,2012]. The production of GA_3 by various fungal species was also observedat an optimum temperature of 30°C. A 25°C was also optimum for GA_3 production by Gibberella fujikuroi. A low GA_3 yield at higher temperature was also recorded for GA_3 production by Aspergillus niger (Rangaswamy V ,2012; Udhandi *et al*., 2010).

Figure -1. Effect of incubation period on gibberellic acid production

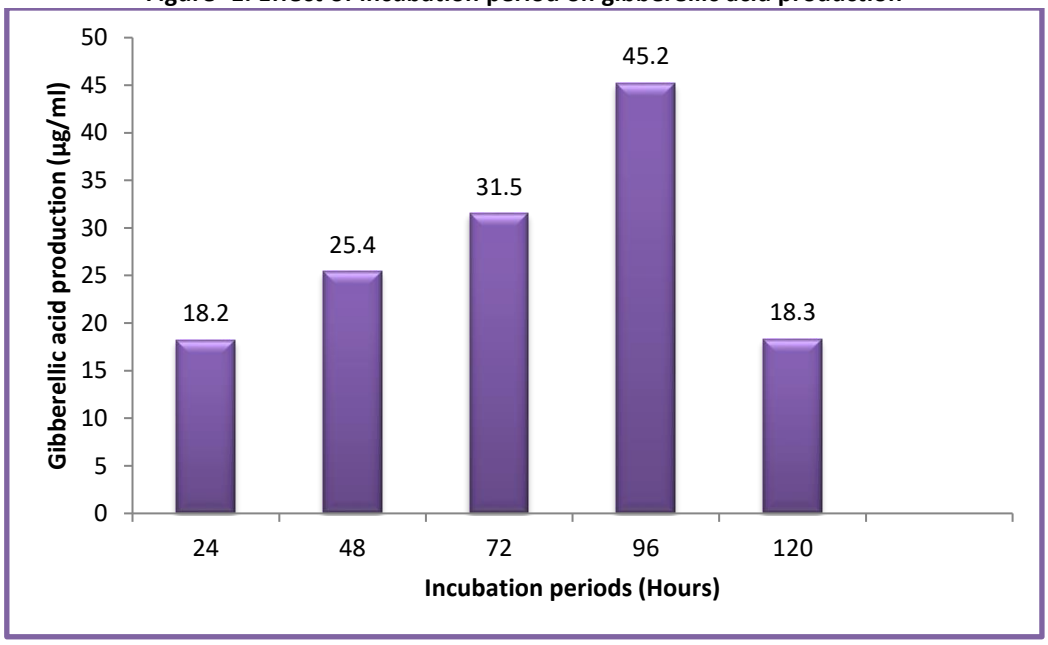


Figure -2. Effect of pH on gibberellic acid production

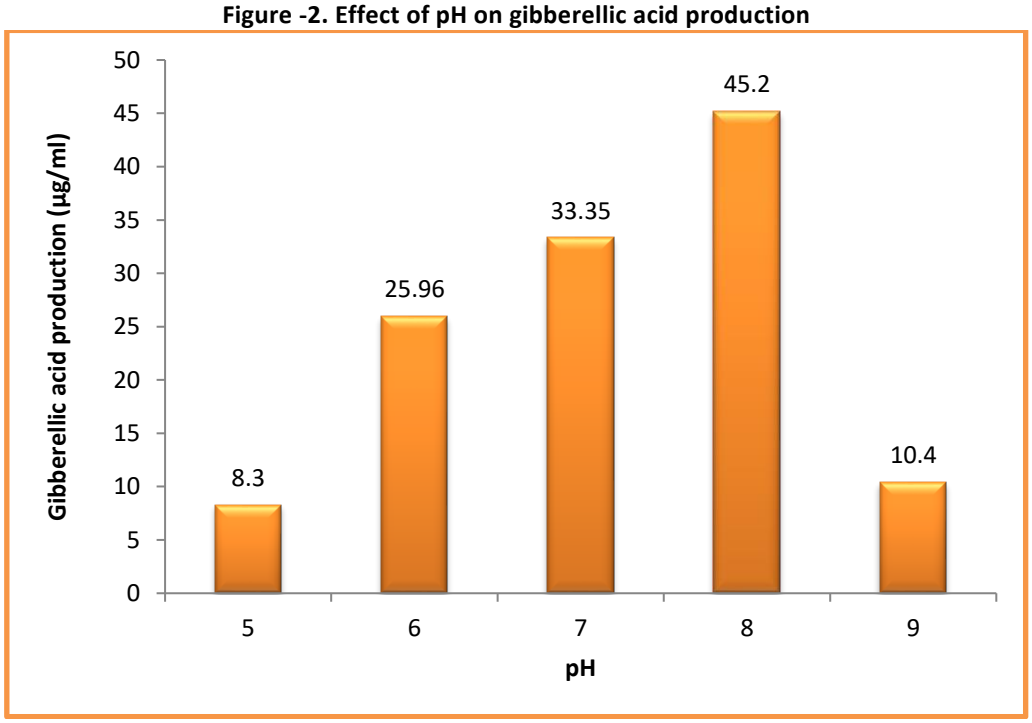
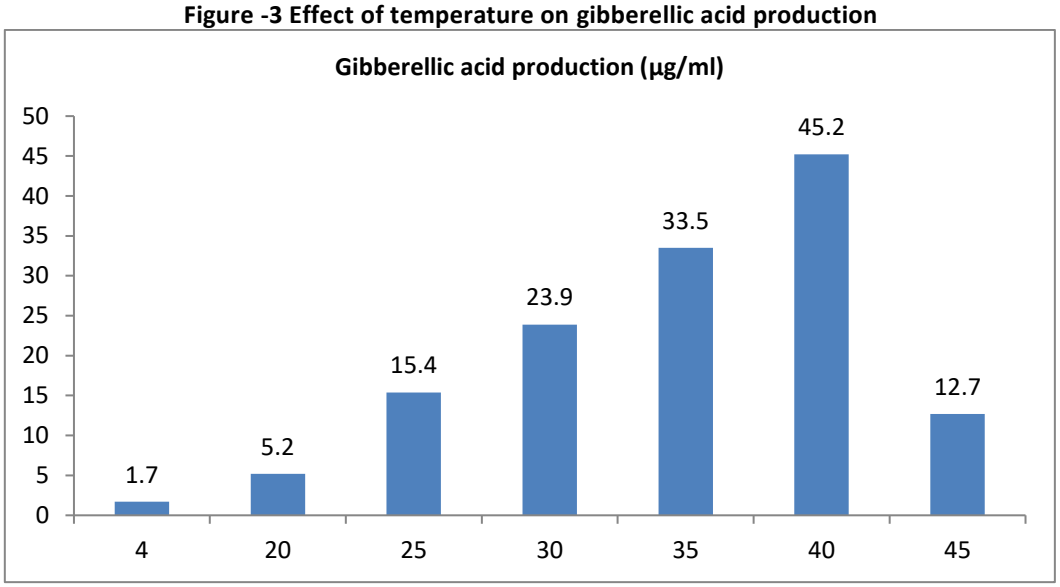


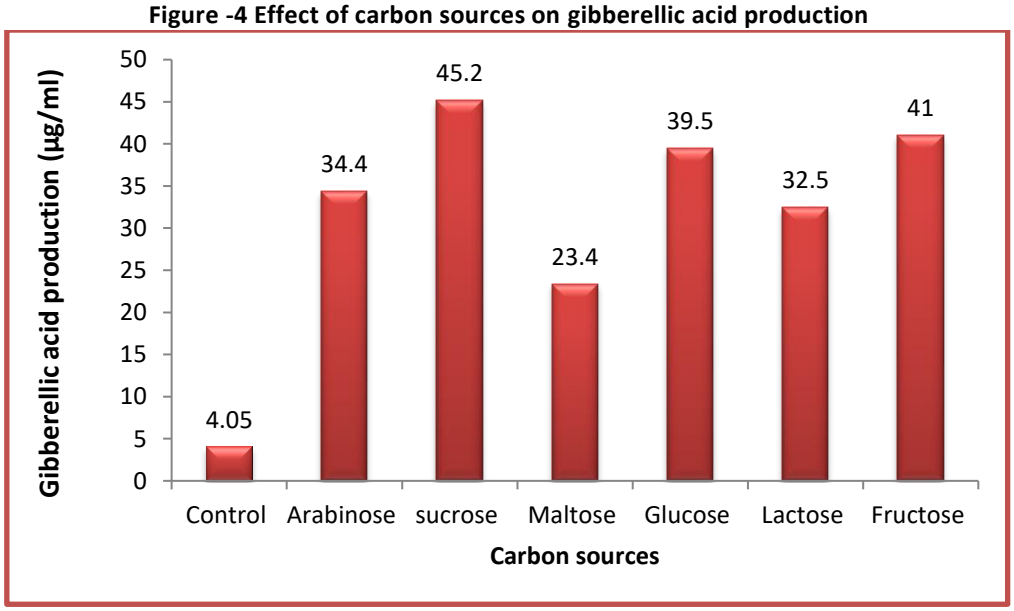
Figure -3 Effect of temperature on gibberellic acid production



Different carbon sources were observed for the gibberellic acid production. Maximum production was obtained in the presence of sucrose and minimum by maltose. A tenfold increase in gibberellic acid production was recorded when compared to control. Sucrose after fructose also showed maximum gibberellic acid production in the medium containing carbon source (Figure-4).

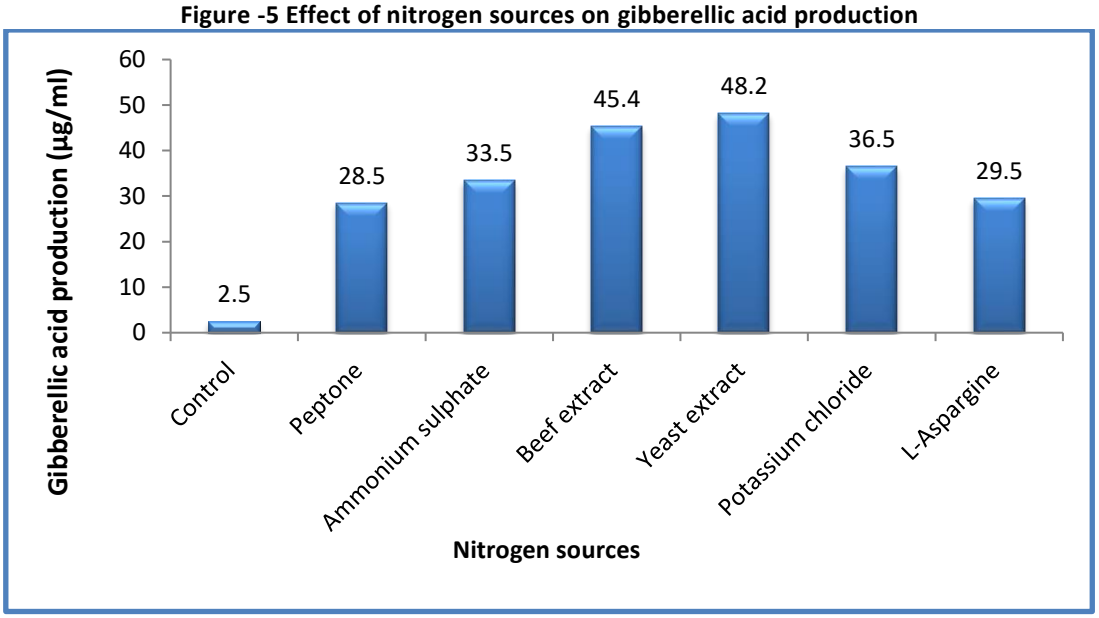
An important yield was seen when ammonium chloride was used as nitrogen source for GA_3 production by Fusarium fujikuroi SG2. Glucose was best carbon source for GA_3 production by Fusarium moniliforme. However, a mixture of glucose and rice flour was necessary to obtain GA_3 production by Fusarium fujikuroi SG2 (Udhandi *et al.*, 2010; Rangaswamy V ,2012; Kobomoje *et al*., 2013; Bilkay *et al*., 2010).

Figure -4 Effect of carbon sources on gibberellic acid production



Various nitrogen sources were observed for the gibberellic acid production. Among them yeast extract (48.2 µg/ml) was recorded to exhibit maximum gibberellic acid production. The other nitrogen sources, beef extract followed by potassium chloride also showed maximum production. The strain DS5 showed maximum gibberellic acid production in the presence of nitrogen sources, compare to control (Figure-5).

Figure -5 Effect of nitrogen sources on gibberellic acid production



CONCLUSION

Optimization studies reveal that the strain DS5 showed highest gibberellic acid production at sucrose and yeast extract. The suitable environmental conditions like 96 h incubation and pH 8.0 at 40^{o}C. Further this potent strain showed the plant growth promoting characteristics, was selected to identification process through 18 S rRNA sequence.

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