# Production of IAA by arbuscular mycorrhizal fungus *Glomus deserticola* and yeast *Pichia fermentans* to improve plant growth

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***Abstract***
*Auxin is a plant hormone which mainly includes indole acetic acid (IAA). It induces root proliferation in plants. The study was designed to evaluate the production of auxin by eukaryotic microbes Pichia fermentans and Glomus deserticola individually and as a co-culture. IAA production was observed from 1st day onwards, which was maximum on 8th day in the co-inoculated flasks (105 µg/ml). Co-inoculation of the cultures could significantly enhance the growth of Cicer arietinum seedling compared with that of non-inoculated seedling.*

*The study also points towards increase in the root associated traits when Cicer arietinum seedlings are treated with crude IAA produced by co-inoculation of yeast and fungus, while significant shoot enhancement was observed by G. deserticola treatment. The present findings revealed P. fermentans and G. deserticola to be efficient organisms for IAA production and point towards a promising system for the in vitro production of IAA.*

**Keywords:** Indole-3-aceticacid; Auxin, *Pichia fermentans*, *Glomus deserticola*, *Cicer arietinum*.

## Introduction

Indole-3-acetic acid (IAA) is the most active and key phytohormone, a product of L-tryptophan metabolism produced in wide range of plants and has major role in plant growth and development such as cell elongation, cell division, cell differentiation and also root initiation[35]. The various environmental factors like temperature and pH influence IAA production[28].

IAA is widely produced by many microorganisms which are mainly dwelling in the rhizosphere of plants. The phylogenetic evidence shows that IAA biosynthesis has evolved independently and natural selection might have favored its production in fungi, bacteria and plants[8].

However, yeast belonging to *Saccharomycetaceae* can be a good choice for the production of such compounds as this group of microbes naturally occurs in fruits and is widely used in fermentation process and also has low risk of pathogenicity[6]. In view of the isolation of *Pichia fermentans* from various plant species, it seems likely that this species may have some significant role in plant growth promotion by producing plant growth regulating hormone[9].

Arbuscular mycorrhizal fungi (AMFs) are obligate symbionts having a non-pathogenic relationship between plant roots and fungal hyphae[7]. Free- living as well as symbiotic PGPR can enhance plant growth directly by providing bio-available mineral nutrients, absorbing trace elements like iron for plants from siderophores, producing phytohormones and lowering plant ethylene levels. Moreover, some rhizosphere bacteria cohabit with AMF and could play a supporting role in the plant-fungus interaction improving plant growth[15].

The fungi producing IAA during plant-fungi interactions suggests that it may use IAA and related compounds to interact with plants for symbiotic strategies leading to plant growth promotion and basal plant defense mechanism[20]. Such mycorrhizal plants usually had high survival and reproductive rate[12] with stronger resistance to biotic and abiotic stress[22]. Hence mycorrhizal association had been widely recognized in both agricultural and ecological systems.

*Cicer arietinum* L. (Chickpea) is a self-pollinated cool-season legume crop which is cholesterol free and good source of carbohydrates, protein, minerals and vitamins[25]. It is consumed all over the world especially in African and Asian Countries, still the majority of soils under chickpea cultivation are sandy and deficient in plant nutrients. Deficiency of mineral nutrients leads to low productivity and economic crises. Due to this, farmers are bound to apply chemical fertilizer for good crop yield. Frequent use of expensive chemical fertilizer pollutes the environment[10]. Therefore, farming communities are shifting from chemical-based agriculture to sustainable organic agriculture.

Therefore, the study involves the influence on IAA production by *Pichia fermentans* and *Glomus deserticola* under laboratory conditions. The product was isolated, purified and checked for its plant growth promoting activity on *Cicer arietinum* seedlings.

## Material and Methods

**Substrate and Organisms:** *Pichia fermentans* (MTCC 189) was procured from MTCC, Chandigarh, India and *Glomus deserticola* (CMCC/AM 2901) was received from the Energy and Resource Institute, India. Potato dextrose agar media (Potatoes infusion from (200 g/L), dextrose (20 g/L)

and agar (15 g/L); pH 6) were used to grow the pure cultures of *P. fermentans* and *G. deserticola*.

**Screening of yeast and Arbuscular mycorrhizal fungus for IAA production:** Flasks containing 50 ml 0.5% (w/v) malt extract were sterilized at 15 lbs/in$^2$ for 15 min and aseptically amended with tryptophan [0.05% (w/v)] (filter sterilized). The flasks were inoculated with *P. fermentans* [1% (v/v)] 12 hrs old actively growing culture or *G. deserticola* (2 mycelial discs of 4 days old culture). To check the effect of co-inoculation, a set of sterilized flasks containing malt extract were inoculated with both the organisms. All the flasks were incubated at 30 °C up to 12 days along with uninoculated control. Two ml aliquot was aseptically taken out from the flask at one day interval and centrifuged at 8000 rpm for 10 min. The supernatant was used for IAA estimation.

**Analytical methods**

**Estimation of IAA:** IAA was quantitatively estimated as per the method described earlier[4]. Briefly, 1 ml of the supernatant was mixed with the equal amount of Salkowski reagent and the OD was read at 530 nm after 30 min of incubation. Uninoculated sample was used as control. The standard IAA was used to prepare a standard curve for quantitative comparison.

**Confirmation of IAA produced by HPLC analysis:** Presence of IAA was further confirmed by HPLC using C18 column (5 µm; 25 x 0.46 cm) with elution performed using the ratio 9:1 of methanol and water containing 0.5% acetic acid with a flow rate of 0.5 ml/min and the detection was monitored at 220 nm at 40 °C.

**Effect of crude IAA on seedling and root development:** Plant growth promotion and root proliferation ability of the crude IAA were assessed on *Cicer arietinum*. The seeds were surface sterilized using 0.1% $HgCl_2$ followed by 4-5 repeated washings with sterilized distilled water. Seeds were germinated for one day and sowed in coco-peat medium treated with crude IAA extract (1ml per seed) obtained by yeast, fungus and their co-inoculation after filtration of suspension in sample flask using Whatmann® filter paper. Seeds were incubated for 10 days at 30 °C followed by 5 days incubation under natural environmental condition, 1 ml sterilized water was added to each flask along with the control. Root length, shoot length, no. of leaves and weight were measured after incubations. All the results were represented as mean ± standard error (n = 20).

## Results and Discussion

**IAA production by yeast and Arbuscular mycorrhizal fungus:** IAA is an essential compound for the growth and development of shoot and roots, many microorganisms including plant growth promoting rizobacteria (PGPR) produce IAA[34]. IAA, a secondary metabolite of the fungus is excreted near the end of the growth phase or during plant dormancy phase by the microorganisms[3].

Therefore, it is expected that this plant regulator production time is long. Mainly through indole-3 -pyruvate acid and tryptamine pathway, most species use tryptophan to produce IAA[30]. IAA can be produced when the external tryptophan becomes available to the fungus[16]. Tryptophan is considered as a precursor for IAA biosynthesis and its addition in culture medium enhances IAA production[18].

It has been reported that IAA production, an important attribute of PGPR is regulated by several factors like strain, concentration of precursor, media components and growth stage etc.[11] Therefore, the conditions were optimized to increase the production of IAA. According to our study both *P. fermentans* and *G. deserticola* produced a significant amount IAA ranging from 23.44 (µg/ml)-105.04 (µg/ml) (Table 1). This indicated *Pichia fermentans* with the ability to produce a significant amount of IAA in a medium supplemented with tryptophan. It revealed the variation in utilization of malt extract, tryptophan for maximum yield. Earlier, *Pichia guilliermondii* and *Hanseniaspora uvarum* produced less than 25 µg/ml when inoculated in yeast extract-dextrose based medium after 7 days of incubation[2].

AMFs were reported to produce IAA ranging from 0.6 (µg/ml)-24 (µg/ml)[27]. Maximum IAA (105.04±13.71 µg/ml) was produced by the co-inoculation of *P. fermentans* and *G. deserticola* on the 8$^{th}$ day (Table 1). Synergism between *Aspergillus niger* and *Glomus deserticola*, promoted considerable increase in phosphorous absorption and growth of *Trifolium repens*[32]. Similarly, dual inoculation of *Glomus aggregatum* and *Bacillus polymyxa* increased biomass in *Cymbopogon martini* var. motia[21].

**Table 1**
**Production of IAA (µg/ml) by *P. fermentans* and *G. deserticola***

| Days of incubation | *P. fermentans* | *G. deserticola* | Co-inoculation |
|---|---|---|---|
| Day-4 | 23.44±0.02 | 29.65±3.12 | 52.87±14.22 |
| Day-6 | 34.58±5.74 | 52.4±0.83 | 87.36±1.42 |
| Day-8 | 25.47±4.34 | 48.32±4.6 | 105.04±13.71 |
| Day-10 | 21.68±2.11 | 42.58±2.17 | 91.5±9.45 |
| Day-12 | 18.33±1.39 | 39.09±2.42 | 70.38±10.20 |
| IAA values are mean of three replicates ± SD. | | | |

**Confirmation of IAA produced by HPLC analysis:** IAA production by *P. fermentans* or *G. deserticola* and their co-culture was confirmed by HPLC using C18 column (5 µm; 25 x 0.46 cm) with elution performed using the ratio 9:1 of methanol and water, containing 0.5% acetic acid with a flow rate of 0.5mL/min and the detection was monitored at 220 nm at 40 °C. HPLC detected the peak at 5.44 min when standard IAA was run (0.1 mg/ml) (Figure 1). A peak comparable to standard IAA confirmed the presence of IAA in samples. An adjacent peak observed might belong to some related indole compound (indole lactic acid) seen during IAA production by fungus *Colletotrichum acutatum*[5].

**Effect of crude extract on seedling growth and plant development:** AMF are known to increase rooting due to the production of plant growth hormones[23]and polyphenolic compounds which decrease auxin oxidation[17]. Increase in shoot and root length in fungal inoculated plants has been reported earlier[24]. Inoculation with AMF generally showed an increase in root hair density of the host plants[33]. Arbuscular mycorrhizal fungi influence plant growth through number of ways[14]. AMF not only absorbs phosphorus faster[31] but also plays an important role in the uptake of water and other plant nutrients which influence the plant growth[29, 1]. All the seeds used in experiments showed germination under the experimental conditions (Figure 2).

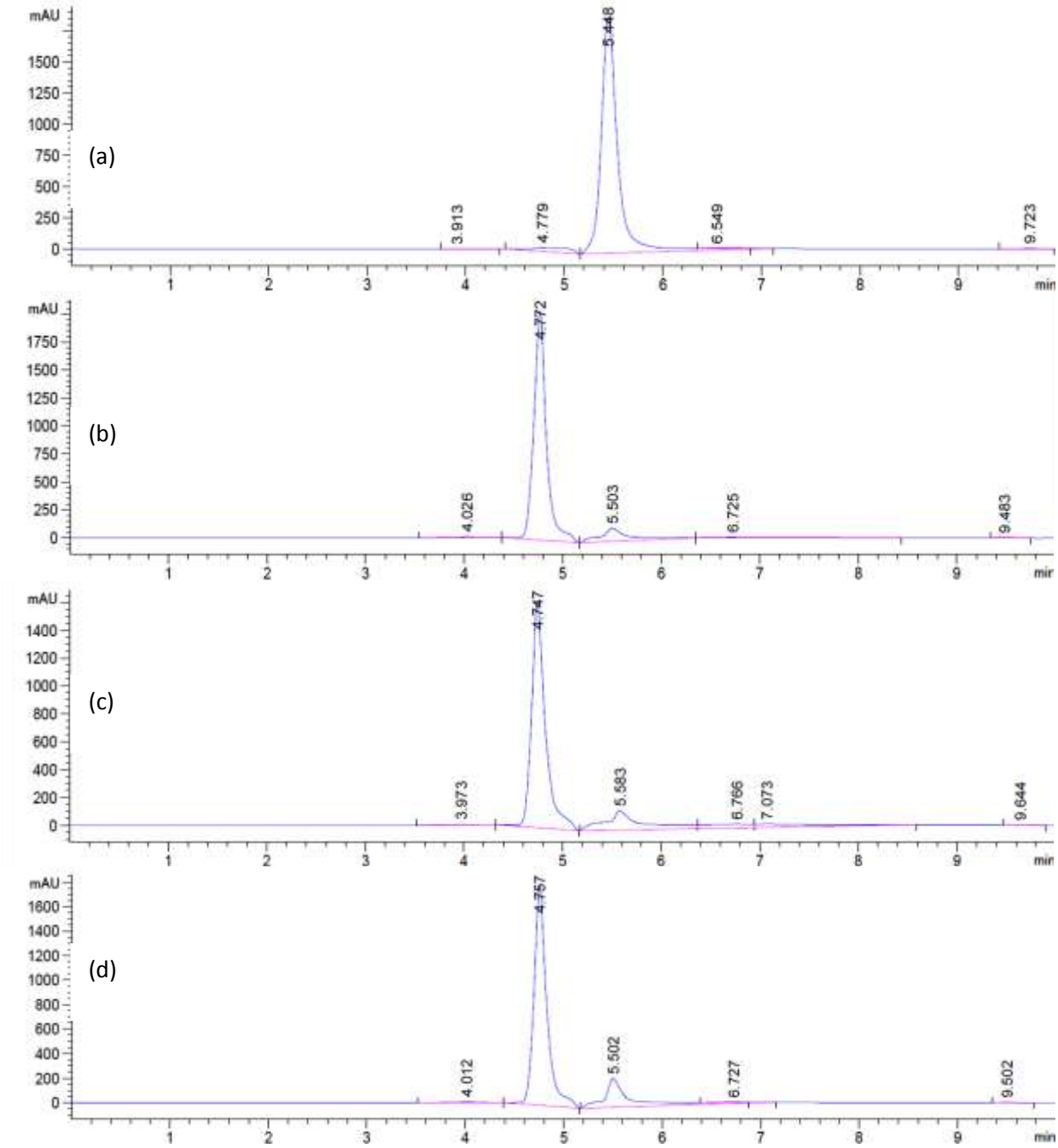


**Figure 1: HPLC profile of (a) Standard IAA, (b) IAA produced by *P. fermentans*, (c) IAA produced by *G. deserticola*, (d) IAA produced by co-inoculation**

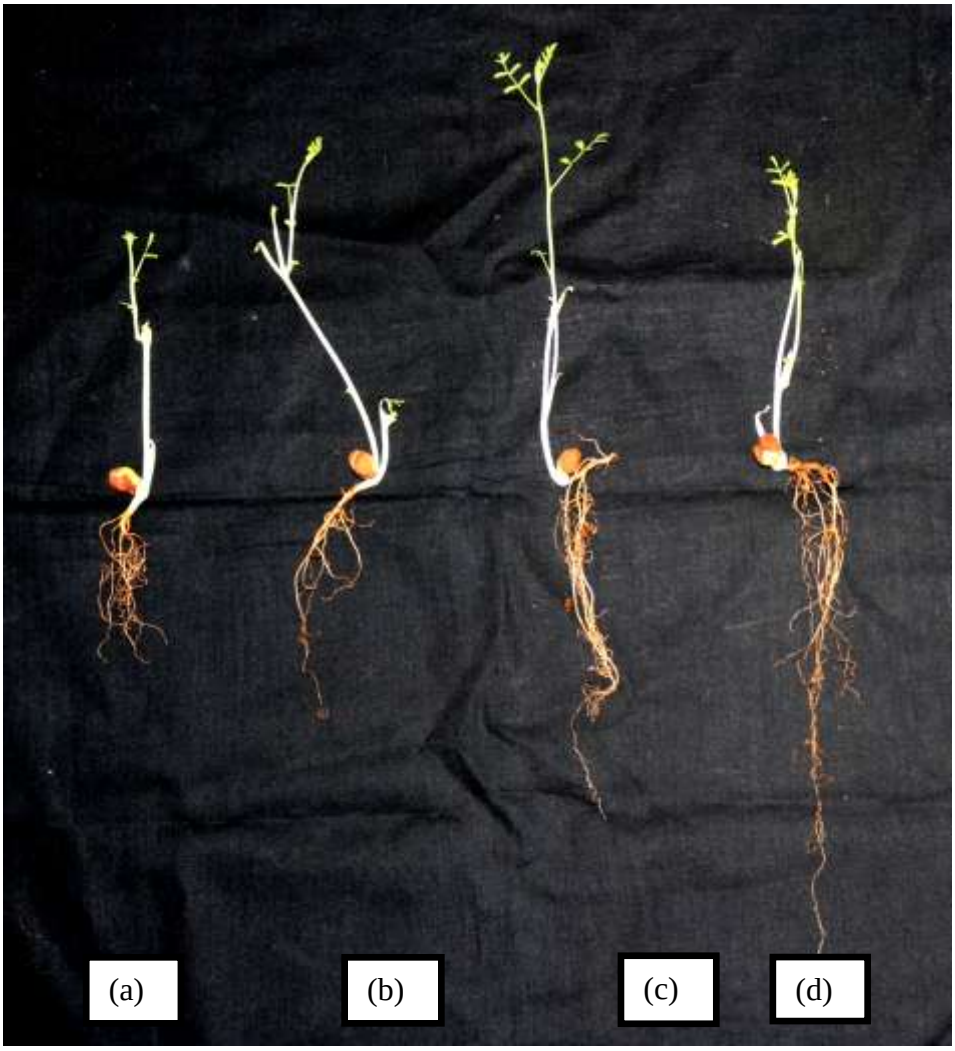


**Figure 2: Growth promotion of *Cicer arietinum* seedling by (a) control, (b) IAA produced by yeast, (c) IAA produced by fungus, (d) IAA produced by co-inoculation**

**Table 2**
**Effect of crude IAA on growth promotion of *Cicer arietinum* seedlings**

| Treatment | Lateral root count | Root length (cm) | Shoot length (cm) | No. of leaves | No. shoot branches | Weight (g) |
|---|---|---|---|---|---|---|
| Control | 8.75±0.08 | 13.75±1.25 | 18±9.47 | 21.75±5.94 | 1.5±2.98 | 1.11±1 |
| *P. fermentans* | 12.5±0.13 | 14.65±4.24 | 13.83±7.21 | 19.75±8.39 | 4.12±3.66 | 1.29±2.6 |
| *G. deserticola* | 11.87±0.15 | 13.12±4.88 | 24.25±6.37 | 40.87±9.53 | 7.62±5.34 | 1.35±1.06 |
| Co-inoculation | 16.75±0.13 | 16±4.46 | 18.08±5.53 | 24.37±5.29 | 6.37±6.71 | 1.29±1.18 |

All values are mean of three replicates ± SD.

In the present study the root associated traits like root length (16±4.46 cm) and lateral root count (16.75±0.13) were significantly enhanced when seeds were treated with IAA produced by the co-inoculation of *P. fermentans* and *G. deserticola* (Table 2) while the shoot associated traits like shoot length (24.25±6.37 cm), no. of leaves (40.87±9.53), shoot branches (7.62±5.34), weight (1.35±1.06 g) were significantly increased by the treatment of seeds with IAA produced by *G. deserticola* alone (Table 2). Earlier, *Glomus mosseae* , *Glomus intraradices* and their co-cultures in presence of *Fusarium solani* also increased the shoot associated traits of chick pea[26]. The influence of *Glomus deserticola* on the increased growth characters of okra (*Abelmoschus esculentus*) has also been reported earlier[19]. *Glomus intraradices* along with *actinomycetes* and Pseudomonas spp. strain enhanced plant growth under normal and water stress condition[13]. Thus, it could be concluded that use of *P. fermentans*, *G. deserticola* or their co-cultures can be a good choice in growth promotion of *Cicer arietinum.*

## Conclusion

Both *P. fermentans* and *G. deserticola* produced significant amount of IAA independently while the production was

enhanced by co-inoculation of both yeast and fungus at the same time. The study also points towards an increment in the root associated traits when *Cicer arietinum* seedlings treated with crude IAA are produced by co-inoculation of yeast and fungus; enhance shoot associated traits when seedlings are treated with crude fungal IAA alone.

Thus the present findings point towards an efficient production of plant hormone by yeast and fungal cultures which may lead to develop a cost effective production of such metabolites and their further use in agriculture field to reduce the negative impact of chemical fertilizers.

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