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Stimulatory effect of phosphate-solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2)

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Abstract

The effect of six phosphate-solubilizing fungi (PSF, two strains of *Aspergillus awamori*, and four of *Penicillium citrinum*) isolated from rhizosphere of various crops, was observed on the growth and seed production of chickpea plants (*Cicer arietinum* L. cv. GPF2) in pot experiments. The phosphate (P) solubilizing activity of PSF in liquid varied from 38 to 760 $\mu g\,ml^{-1}$ for tricalcium phosphate (TCP) and 28–248 $\mu g\,ml^{-1}$ for mussoorie rock phosphate (MRP). All PSF isolates were biocompatible and produced growth-promoting hormone, Indole acetic acid (IAA), varying in concentration from 2.5 to 9.8 $\mu g\,ml^{-1}$. Of the various pot experiments carried out in green house, maximum stimulatory effect on chickpea plants growth was observed by inoculation of two *A. awamori* strains. This treatment resulted in 7–12% increase in shoot height, nearly three-fold increase in seed number and two-fold increase in seeds weight as compared to the control (un-inoculated) plants. Inoculation of four strains of *P. citrinum* exhibited lesser stimulatory effect. It showed 7% increase in shoot height, two-fold increase in seed number and 87% increase in seeds weight as compared to the control plants. However, a consortium of all the six fungal isolates showed no stimulatory effect on chickpea plants growth.

Keywords: *Aspergillus awamori*; *Penicillium citrinum*; Phosphate-solubilizing microorganisms; RAPD; Indole acetic acid; Chickpea

1. Introduction

Phosphorus is one of the major essential mineral fertilizers and is world's second largest agricultural chemical required by plant for its growth and development. Majority of the inorganic phosphorus applied to soil as a chemical fertilizer is rapidly fixed as insoluble forms (phosphates of iron, aluminum and calcium) and thus become unavailable to plants (Altomare et al., 1999). In addition, chemical fertilizers are costly and have adverse effect on the soil fertility (Vassilev and Vassilev, 2003).

Soil microbes have the ability to convert fixed form of phosphorus (in soil) to soluble forms that can be easily taken up by plants (Rodriguez and Fraga, 1999). High proportions of these phosphate-solubilizing microorganisms (PSMs) are concentrated in the rhizosphere of plants (Vesquez et al., 2000). Many studies have shown an increase in growth and P-uptake by plants through the inoculation of PSMs in pot experiments (Omar, 1998; Vassilev et al., 2006) and under field conditions (De Freitas et al., 1997; Duponnois et al., 2005; Valverde et al., 2006). These PSMs can also increase the growth of plants by other mechanisms i.e. production of phyto hormones such as Indole acetic acid (IAA) (Patten and Glick, 2002; Mehnaz and Lazarovits, 2006) which are plant growth promoters.

Although PSMs occur in soil, usually their number is not high enough to compete with other microorganisms commonly established in the rhizosphere. Therefore, inoculation of plants by target microorganisms at higher concentration has beneficial effect. Moreover, only one type of microorganism may not be effective for plant growth enhancement and crop yield because of its inability to: (a) compete with native microorganisms and

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(b) colonize properly in new soil environment. Hence, a consortium of PSMs is preferred for soil inoculations so that at least one type of PSM will be able to establish in the soil.

Chickpea is the third most important legume crop in the world and act as rich supplement to cereal diet in developing countries. The aim of the present study was to isolate phosphate-solubilizing fungi (PSF) from rhizosphere of main crop plants and examine their effect on the yield of chickpea (*Cicer arietinum* L. cv. GPF2) seeds in pot experiments. This plant is an improved variety developed at the Panjab Agricultural University, Ludhiana, India and is being widely used in north-western part of India. So far no study has been carried out on the use of PSF consortium on *C. arietinum* L. cv. GPF2, hence this study was undertaken. The experiments were carried out in glass house during the winter season (November to April, 2005–2006).

2. Materials and methods

2.1. Isolation and screening

The fungal strains were isolated from rhizosphere soil samples collected from the various agricultural fields located around Chandigarh, India. Hundred microliter aliquots of various serial dilutions of soil samples were plated on to Pikovskaya's medium (PVK, 1948) supplemented with ampicillin (50 $\mu g\,ml^{-1}$) and streptomycin (30 $\mu g\,ml^{-1}$), and incubated at 30 °C upto 6 d. The PSF showing zone of clearance around the fungal colonies were selected and maintained on Potato Dextrose Agar (PDA) slants at 4 °C.

2.2. Identification of fungi

Fungal isolates were identified up to genus level based on the colony morphology on PVK agar medium and lactophenol cotton blue staining technique which determines the type of reproductive mycelium i.e. conidiophore. Species identification was carried out at Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India based on their colony morphology on different media (Chapek yeast extract agar, malt extract agar, 25% glycerol nitrate agar) at different incubation temperature (5, 25, and 37 °C), spore characteristics and microscopic examination. The strains were deposited MTCC and MTCC accession numbers are given in Table 1.

2.3. Quantitative assay of P-solubilization

All fungal isolates were tested for their ability to solubilize inorganic phosphates, TCP (tricalcium phosphate) and MRP (mussoorie rock phosphate). MRP was obtained from M/S Pyrites, Phosphates and Chemicals Ltd., Noida, India as 100-mesh size powder. It contained following components in %: CaO, 38.50; P_2O_5, 21.20; F, 2.30; CO_3, 13.80; Na_2O, 0.17; MgO, 5.60; K_2O, 0.25; Al_2O_3, 0.73; SiO_2, 6.60; Fe_2O_3, 4.41; sulfide–sulfur, 4.00; organic-C, 1.14; chlorides, 0.015; SO_4–S, 0.10; neutral ammonium citrate (P_2O_5), 2.20.

Table 1
MTCC[a] accession number, amount of Indole acetic acid (IAA) and dry mycelium mass produced by selected fungal isolates

Strains	MTCC accession number	IAA ($\mu g\,ml^{-1}$)[b]	Dry mycelium mass ($mg\,ml^{-1}$)
Aspergillus awamori VQ2	8002	2.5 ± 0.01	13.5 ± 0.09
Aspergillus awamori VHI	8003	3.0 ± 0.01	12.3 ± 0.07
Penicillium citrinum VPT	8004	9.8 ± 0	14.1 ± 0.01
Penicillium citrinum VU1	8005	9.5 ± 0.02	11.5 ± 0.01
Penicillium citrinum VR50	8006	6.5 ± 0.01	13.9 ± 0.02
Penicillium citrinum VPC	8007	6.0 ± 0.01	14.5 ± 0.04

Note: Dry mycelium mass was estimated on 4 d, the mycelium mats were removed, washed with distilled water, dried for 2 d in oven at 70 °C and weighed.

[a]MTCC is Microbial Type Culture Collection & Gene bank, at Institute of Microbial Technology, Chandigarh, India.

[b]Data is mean of three replicates, ± indicates standard deviation.

The isolates were grown in 250 ml conical flasks containing 100 ml liquid medium. The composition of the medium ($g\,l^{-1}$) was, yeast extract, 0.50; dextrose, 10.0; TCP/MRP, 5.0; $(NH_4)_2SO_4$, 0.50; KCl, 0.20; $Mg_2SO_4.7H_2O$, 0.10; $Mn_2SO_4.H_2O$, 0.0001; $Fe_2SO_4.7H_2O$, 0.0001. The pH of the medium was adjusted to 7.0. Five-millimeter mycelial discs of each isolate from actively growing 72 h old culture (grown on PDA) were added as inoculum. The flasks were incubated at 30 °C for 10 d on a rotary shaker at 150 rev min^{-1}. After 2, 4, 6, 8, 10 d of incubation, a 5 ml culture was withdrawn from each flask. The cultures were centrifuged (Sigma 103456 cooling centrifuge, Germany) at 10,000*g* for 5 min and supernatant filtered through Whatmann No. 1 filter paper. The pH of the supernatant was measured using pH meter (HPG G-2001, India). The phosphorus in solution was determined using colorimetric (660 nm, Jenway 6305 spectrophotometer, England) chlorostannous reduced molybdo phosphoric acid blue method of Jackson (1973). All the data are mean of three replicates. At end of incubation period the mycelia mates were gently removed, washed with distilled water and dried to estimate mycelium dry mass.

2.4. Biocompatibility assay

Biocompatibility assay was conducted to study the antagonistic properties of PSF. A single fungal stain was streaked as a straight line in the center of PVK agar plate. Cultures to be tested were streaked perpendicularly across the initial culture and incubated at 30 °C for 48–96 h. Lack of microbial growth (zone of inhibition) at the intersections was indicative of the bio-incompatibility of the cultures.