Environmental Sustainability (2018) 1:245–252
https://doi.org/10.1007/s42398-018-0025-z

ORIGINAL ARTICLE

Plant growth promoting potential of *Aspergillus* sp. NPF7, isolated from wheat rhizosphere in South Gujarat, India

N. D. Pandya[1] · P. V. Desai[2] · H. P. Jadhav[3] · R. Z. Sayyed[3]

Received: 24 March 2018 / Revised: 3 September 2018 / Accepted: 5 September 2018 / Published online: 10 September 2018

Abstract

Many plant growth promoting fungi (PGPF) that perform well under laboratory conditions, fail to do so under natural conditions. Only those PGPF that are acclimatized to the local agro-climatic conditions, can efficiently grow as compared to those that are brought from different agroclimatic conditions. PGPF isolated from local rhizosphere soil and used as locally adapted bio-inoculants are expected to give better performance. Production of phytohormones is an inherent property in all groups of PGPF. Here we report gibberellic acid (GA), indole acetic acid (IAA), siderophore producing and phosphate (P) solubilizing *Aspergillus* sp. NPF7 isolated from wheat rhizosphere as PGPF for wheat and chickpea. In total 12 fungal isolates were obtained from the different rhizosphere of four local crops in the vicinity of Surat city, Gujarat, India. Isolate, NPF7 produced copious amount of IAA (96 μgmL^{-1}), GA (184.11 μgmL^{-1}) and siderophore (87% SU) and efficiently solubilized phosphate (790 mgL^{-1}). It was identified as *Aspergillus* sp. on the basis of homology of its 18S rDNA sequencing. Inoculation of plants with *Aspergillus* sp. promoted growth of wheat and chickpea significantly, enhanced germination index and root and shoot length over the control (uninoculated seeds). *Aspergillus* sp. NPF7 producing multiple plant growth promoting metabolites can be useful bioinoculant for wheat and chickpea.

Keywords *Aspergillus* sp. · Indole acetic acid · Gibberellic acid · Siderophore · Phosphate solubilization

Introduction

Phytohormones are dynamic signaling molecules required in very low concentration for a variety of physiological processes in plants (Khan et al. 2012). Among the various phytohormones, gibberellic acid (GA) and indole acetic acid (IAA) play a vital role in plant growth promotion. Exogenous supply of these phytohormones through plant growth promoting fungi (PGPF) help the plant to grow and withstand normal as well as stress conditions such as salinity, drought, and high or low temperature (Davičre and Achard 2013; Khan et al. 2013). In addition to the endogenous pool of phytohormones, plants also need an exogenous supply of phytohormones for their overall growth and vigor (Lubna et al. 2018a; Gurdeep Kaur and Reddy 2017).

Members of *Aspergillus* sp. are known to supply a variety of phytohormones along with other plant beneficial metabolites such as siderophore and phosphate solubilization. Phytohormone production by these fungi has been suggested as one of the crucial mechanisms for plant growth promotion and stimulation of induced systemic resistance (ISR) in the plant thus making the plant resistant to a variety of diseases (Yamaguchi 2008; Kang et al. 2009). Phytohormone producing PGPF also increase the endogenous level of phytohormones and isoflavonoids in plants that imparts ISR to plants. In addition to the production of phytohormones, many PGPF are known to provide iron and phosphorus (P) nutrition (Sharma et al. 2013, 2016) to the plants through the production of siderophore and phytase/phosphatase respectively. Siderophore producing fungi provide iron nutrition as well as help in the suppression of plant pathogens by depriving them for available iron (Sayyed et al. 2013; Shaikh et al. 2014; Patel et al. 2016).

✉ R. Z. Sayyed
sayyedrz@gmail.com

[1] Department of Microbiology, Arts, Science, and Commerce College, Kamrej Cross Road, Surat, Gujarat 394 185, India

[2] Department of Biosciences, Veer Narmada South Gujarat University, Surat, Gujarat 395 007, India

[3] Department of Microbiology, PSGVP Mandal's, Arts, Science, and Commerce College, Shahada, Maharashtra 425 409, India

Although plant growth promoting potential of *Aspergillus* sp. is well known, reports on the production of phytohormones and siderophore by this fungus are scarce (Khan et al. 2008; Hamayun et al. 2009) moreover, significant differences exist in the performance of PGPF under controlled laboratory conditions and in natural conditions (You et al. 2015). Only those strains that are adapted to natural conditions can perform well under natural soil conditions and thus can be used as effective bioinoculants (Pereg and McMillan 2015; Albermann et al. 2013; Cassán et al. 2013). Hence the present study was aimed to isolate *Aspergillus* sp. from local rhizosphere, evaluate the production of multiple plant growth promoting (MPGP) traits and plant growth promoting potential in wheat and chickpea under local conditions.

Materials and methods

Isolation of fungi from the rhizosphere

Isolation of fungi from the rhizosphere of crop plants viz. rice (*Oryza sativa*), sugarcane (*Saccharum officinarum*), wheat (*Triticum aestivum*) and chickpea (*Cicer arietinum*) was carried out. Uprooted plants were brought to the laboratory in polythene bags and the soil particles loosely adhered to the roots were gently teased out and used for isolation of fungi from the rhizosphere. Each soil sample (1 g) was individually mixed in 100 mL sterile distilled water with constant shaking at 80 rpm for 20 min to get the rhizosphere suspension. Similarly, roots with tightly adhering soil particles were cut into small pieces and 1 g of these root pieces were vigorously mixed in sterile 100 mL distilled water with constant shaking at 80 rpm for 20 min to get the rhizoplane suspension. These rhizosphere and rhizoplane suspensions were serially diluted up to the fifth dilution and 0.1 mL from each dilution was grown on Sabouraud's agar containing gL^{-1}; peptone 10.0; glucose 40.0; NaCl 5.0; agar 2.5 g; supplemented with 70 µg mL^{-1} of streptomycin at 30 °C for 3–7 days and observed for fungal growth. Morphologically distinct fungal colonies from each plate were purified by repeated sub-culturing on potato dextrose agar (PDA) and each culture was maintained on PDA and stored at 4 °C until used.

Screening for production of plant growth promoting traits

A total of 12 fungal isolates were screened for their ability to produce IAA, for this purpose, a spore suspension of the fully grown fungal culture of each fungus was individually inoculated in Sabouraud's broth and incubated at 30 ± 2 °C at 100 rpm for 3 days. After incubation, each broth was centrifuged at 10,000 rpm for 20 min, in 2 mL of supernatant, two drops of orthophosphoric acid, 4 mL of the Salkowski's reagent and 1 mL of 0.5 M $FeCl_3$ solution was added and incubated at 28 °C for 30 min and observed for development of red color. The intensity of red color developed within 30 min was measured spectrophotometrically at 530 nm (Brick et al. 1991; Unyayar et al. 1996) with the un-inoculated medium as a reference. Amount of IAA produced was calculated from the calibration curve of IAA (Sigma-Aldrich, CA, USA).

A total of 12 fungi obtained from rhizosphere as above were screened for their ability to produce GA, for this purpose, a spore suspension of the fully grown culture of each fungus was individually inoculated in Sabouraud's broth and incubated at 30 ± 2 °C at 100 rpm for 3 days. After incubation, the broth was centrifuged at 10,000 rpm for 20 min and pH was of culture supernatant was adjusted to 2.5 by using 3.75 N HCl. GA from the cell-free supernatant was extracted with ethyl acetate and $NaHCO_3$ (1:1) (Holbrook et al. 1961; Taiwo et al. 2017). The amount of GA in the ethyl acetate phase was measured spectrophotometrically at 254 nm with a UV visible spectrophotometer (Shimadzu, Japan) with the un-inoculated medium as a reference (Berryos et al. 2004). Amount of GA produced was calculated from a calibration curve prepared with standard GA (Sigma-Aldrich, CA, USA).

The ability of fungal isolates to produce siderophores was checked by separately growing each culture on chrome azurol sulfonate (CAS) agar plate containing gL^{-1}; K_2HPO_4 3.0; KH_2PO_4 2.0; $MgSO_47H_2O$ 0.2; NH_4SO_4 1.0; succinic acid 4.0; agar 2.5 at 30 °C for 48–72 h (Milagres et al. 1999; Patel et al. 2016). After incubation, plates were observed for change in color of the medium from blue to orange-red around the colony (Patel et al. 2018).

Production of siderophore was carried out by shake flask method in succinic acid medium (SAM) containing gL^{-1}; K_2HPO_4 3.0; KH_2PO_4 2.0; $MgSO_47H_2O$ 0.2; NH_4SO_4 1.0; succinic acid, 4.0 (Meyer and Abdallah 1978). For this purpose, a spore suspension of each fungal culture was separately grown in SAM at 30 °C at 120 rpm for 48–72 h. After incubation, each broth was centrifuged at 10,000 rpm for 20 min, detection of siderophore from each cell-free supernatant was carried out by the CAS test (Schwyn and Neilands 1987). In this, 0.5 mL of cell-free supernatant was added to 0.5 mL of CAS solution and observed for a color change from blue to orange. Quantitative estimation of siderophore from each supernatant was carried out by CAS shuttle assay (Payne 1994). In this assay, 1.5 mL of cell-free supernatant was added to 1.5 mL of CAS solution and color development was read at 630 nm with uninoculated broth as a reference. Amount of siderophore from succinic acid broth was estimated by using the following formula and siderophore content was expressed as percent siderophore unit (SU):

$$\%SU = \frac{Ar - As}{Ar} \times 100,$$

where, Ar is the absorbance of the reference at 630 nm (CAS reagent), As is the absorbance of the sample at 630 nm.

The ability of each fungal isolate to solubilize phosphate was checked on Pikovoskaya's agar (PKA) as per the method of Gaur (1990). For this purpose spore suspension of the fully grown culture of each fungus was individually grown on PKA containing; 0.5% calcium phosphate as the inorganic/insoluble phosphate at 30 ± 2 °C for 7 days and daily observed for the zone of phosphate solubilization. Colonies showing a zone of phosphate solubilization were picked up, purified and maintained on PKA at 4 °C.

For selection of efficient phosphate solubilizing fungi (PSF), spore suspension of each fungal culture was separately grown in Pikovoskaya's broth (PKB) at 30 °C with constant shaking at 120 rpm for 7 days. After incubation, the broth was centrifuged at 10,000 rpm for 20 min and cell free supernatant was assayed for quantitative estimation of phosphate solubilization by using the molybdenum blue method (Murphy and Riley 1962). The extent of phosphate solubilization on PKA and in PKB was taken as criteria for selection of potent PSF. Isolate showing maximum zone of phosphate solubilization on PKA and degree of phosphate solubilization in PKA was selected as efficient PSF.

Identification of potential fungal isolate

Potential fungal isolate NPF7 was identified based on homology of its 18S rDNA sequences with the available sequences at GenBank. The chromosomal DNA of isolate NPF7 was isolated as described by Sambrook and Russel (2001). Complete 18S rDNAs were PCR amplified as per the method of Adachi et al. (1996) using forward primer 5′CTTGGT CATTTAGAGGAAGTAA3′ and reverse primer 5′TTGGTC ATAGAGGAAGTAA3′ that were complementary to the 18S rDNA of fungus. The nucleotide sequence homology of this fungal isolate was matched with the DNA sequences available in the database with the help of the BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/). The closely related nucleotide sequences were aligned by CLUSTAL-W and the neighbor-joining tree method by using MEGA version 4.0 software.

Test for aflatoxin production

In order to assure the absence of aflatoxin production by *Aspergillus* sp. NPF7, a spore suspension of *Aspergillus* sp. NPF7 and aflatoxin-producing standard culture of *A. flavus* NRRL 3251 were separately grown in glucose yeast extract agar (GYEA) at 28 °C in dark for 3 days. After incubation, Petri plates were placed upside down on black paper and were irradiated with longer wavelength UV light (365 nm) (Raed et al. 2016). Absorption of UV light by fungal colonies was taken as criteria for aflatoxin production.

Plant growth promotion studies

Plant growth promoting ability of *Aspergillus* sp. NPF7 was evaluated in wheat (*T. aestivum* L.) and chickpea (*C. arietinum*). For this purpose wheat (Sharbati-Rajwadi variety) and chickpea (GG3-Gujarat Gram 3 variety) seeds were surface sterilized with 0.1% aqueous solution of $HgCl_2$ (Mineo 1990), treated with 100 ppm uniconazole (Khan et al. 2008) and germinated in sterilized Petri plates lined with absorbent cotton moistened with double distilled water. Seeds were sprayed uniformly with 50 mM of uniconazole and culture broth of *Aspergillus* sp. NPF7 containing MPGP traits, while seeds treated with water were taken as control (2.4 mL of each solution per seed) and incubated for 12 h (Saito et al. 2006). Uniconazole was added to block in vivo synthesis of GA by seeds during seed germination. Wheat and chickpea plants were grown in pot controlled environment chamber for 15 days and observed for an increase in germination, root length and shoot length. Seeds of wheat and chickpea plants sprayed with uniconazole and sterile distilled water were used as control (Lubna et al. 2018b).

Statistical analysis

Data obtained were statistically analyzed and the mean of three replicates was taken into consideration. Each mean value was analyzed by using the Student's *t* test and the results were expressed as the values generated from the Student's *t* test. Values of $P \leq 0.05$ were taken as statistically significant (Parker 1979).

Results

Isolation of fungi from the rhizosphere

A total of 12 morphologically distinct fungi were obtained from various soil samples collected from rice, sugarcane, wheat and chickpea rhizosphere and rhizoplane. These isolates were initially labeled as NPF 1 to NPF12.

Screening for production of plant growth promoting traits

Most of the fungal cultures produced IAA in the range of 6–96 µg mL^{-1}. However, isolate NPF7 produced copious amount (96 µg mL^{-1}) of IAA while isolate NPF11 produced the lowest amount (19.56 µgmL^{-1}) of IAA (Table 1).

Table 1 Plant growth promoting traits of the isolates

Isolates	Host plant	IAA production (μg mL^{-1})	GA production (μg mL^{-1})	Siderophore production (%SU)	P solubilization (mgL^{-1})
NPF 1	Rice plant	6.0 (0.012)	Nd	32.00 (0.032)	101 (0.042)
NPF 2	Chickpea plant	68 (0.021)	108.2 (0.012)	57.00 (0.033)	309 (0.029)
NPF 3	Rice plant	28 (0.015)	Nd	31.10 (0.039)	121 (0.030)
NPF 4	Sugar cane plant	Nd	19.5 (0.039)	41.21 (0.037)	143 (0.027)
NPF 5	Sugar cane plant	28 (0.032)	Nd	40.12 (0.040)	138 (0.028)
NPF 6	Sugar cane plant	45 (0.024)	97.6 (0.045)	39.31 (0.039)	152 (0.025)
NPF 7	Wheat plant	96 (0.087)	184.1(0.093)	87.00 (0.079)	890 (0.088)
NPF 8	Sugar cane plant	20 (0.017)	60.5 (0.049)	42.11 (0.041)	145 (0.031)
NPF 9	Wheat plant	38 (0.016)	73.7 (0.039)	53.11 (0.039)	413 (0.024)
NPF 10	Chickpea plant	Nd	48.0 (0.029)	49.21 (0.029)	301 (0.021)
NPF 11	Chickpea plant	14 (0.09)	58.5 (0.042)	52.31(0.026)	294 (0.012)
NPF 12	Sugar cane plant	Nd	Nd	43.18 (0.033)	168 (0.22)

Values were taken to be statistically significant at $P \leq 0.05$

Values are the mean of three replicates

Values in parenthesis are the values generated by comparing control with test by the Students *t* test

Nd not detected

Under flask conditions, all 12 fungal isolates produced a varying amount of GA ranging from 19.56 to 184.11 μgmL^{-1} in Sabouraud's medium. Isolate NPF7 produced maximum (184.11 μgmL^{-1}) amount of GA while isolate NPF4 produced the lowest amount (19.56 μgmL^{-1}) (Table 1).

After 72 h incubation, CAS agar inoculated with *Aspergillus* sp. NPF7 turned orange from blue indicating production of siderophore. Siderophore producing ability of isolate was further confirmed by CAS test, where the addition of CAS solution to cell-free supernatant changed the blue color of CAS to orange color vis-a-vis no color change was observed in un-inoculated CAS agar and SAM. All isolates produced varying amount of siderophore in the range from 32 to 87% SU, however, maximum (87% SU) was produced by isolate NPF7 (Table 1).

All fungal isolates except NPF1, NPF3 and NPF12 exhibited phosphate solubilization potential on PKA; a marked zone of phosphate solubilization was observed around the phosphate solubilizing colony. Isolate *Aspergillus* sp. NPF7 produced a larger zone of phosphate solubilization on PKA agar and solubilized maximum phosphate (890 mgmL^{-1}) on the 7th day of incubation. Among all the isolates, isolate NPF7 gave maximum P solubilization and thus was selected as efficient PSF.

Identification of potential fungal isolate

On the basis of 18S rDNA sequences homology with the available sequences at GenBank, isolate NPF7 showed higher homology to *A. flavus* CBS 14169, *A. flavus* CBS 14170, *A. flavus* 1195 and *A. parasiticus* BAB-81 (Fig. 1). Therefore it was identified as *Aspergillus* sp. and the 18S rDNA sequence of the isolate was submitted to NCBI GenBank in the name *Aspergillus* sp. NPF7 under the accession no. KF477095.

Test for aflatoxin production

The growth of *Aspergillus* sp. NPF7 on GYEA when observed under UV photography showed white colonies, reflecting the non-absorption of UV light indicative of the absence of aflatoxin production by isolate while the standard culture of *A. flavus* NRRL 3251 showed grey-black colonies, reflecting absorption of UV light indicative of aflatoxin production.

Plant growth promotion studies

In order to correlate the production of MPGP traits and plant growth promoting ability of *Aspergillus* sp. NPF7, its potential was assayed in wheat and chickpea plants. Inoculation of wheat and chickpea seeds with *Aspergillus* sp. NPF7 exhibited a significant increase in germination rate, root length and shoot length (Table 2) as compared to control seedlings. A 26.44% increase in seed germination, 12.66% increase in root length and 3.13% increase in shoot length was evident in wheat while in chickpea seeds, 41.50% increase in seed germination, 5.34% increase in root length and 5.12% increase in shoot length was evident over the untreated (control) seeds.

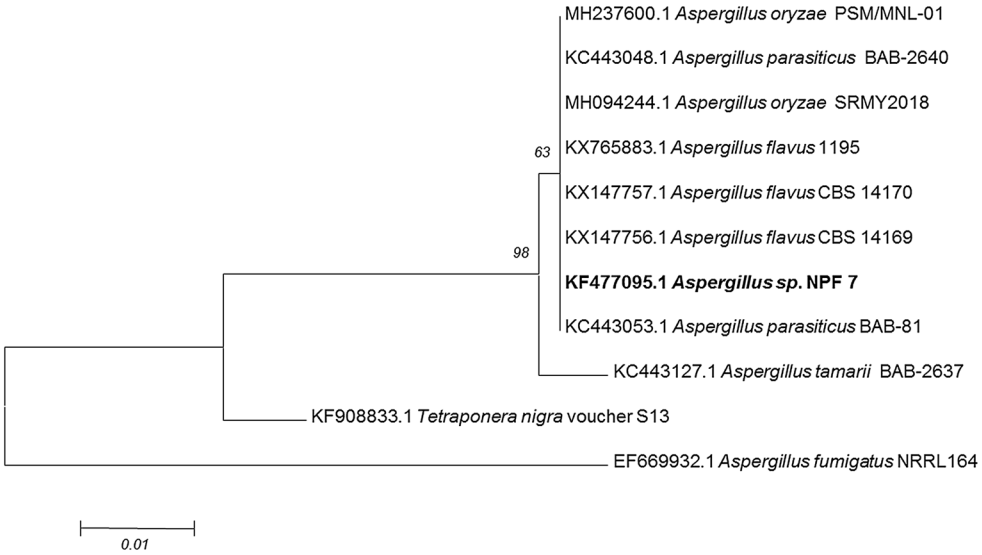

Fig. 1 Phylogenetic tree analysis of *Aspergillus* sp. NPF 7

Table 2 Effect of *Aspergillus* sp. NPF7 inoculation on wheat and chickpea

Crops	Treatment	Shoot length (cm)	Root length (mm)	Germination index (%)
Wheat	Control	16.42 (0.36)^{NS}	33.33 (0.16)^{NS}	60.22 (0.51)^{NS}
	Aspergillus sp. NPF7	35.55 (0.012)	68.67 (0.011)	106.66 (0.051)
Chickpea	Control	17.33 (0.27)^{NS}	102.67 (0.17)	69.11 (0.41)^{NS}
	Aspergillus sp. NPF7	32.53 (0.024)	115.33 (0.09)	117.61 (0.21)

Values were taken to be statistically significant at $P \leq 0.05$

Values are the mean of three replicates

Values in parenthesis are the values generated by comparing control with test by the Students *t* test

NS statistically non-significant at $P \leq 0.05$

Discussion

The occurrence of diverse PGPF in rhizosphere and rhizoplane is attributed to the nutrient richness of the environment. Root exudates such as sugars, amino acids, vitamins, organic acids serve as "rhizodeposition" making the rhizosphere rich in nutrients and thus creating a unique environment for growth and multiplication of soil microorganisms (Prashar et al. 2014). Microorganisms growing in the vicinity of the root zone exhibit various plant growth promoting attributes such as the production of phytohormones, siderophore, and P solubilization. These metabolites help in plant growth promotion and stress tolerance (Jadhav and Sayyed 2016; Jadhav et al. 2017).

Production of copious amounts of IAA and GA by *Aspergillus* sp. NPF7 is attributed to the inherent property of all PGPF to produce various phytohormones. You et al. (2015) have reported the production of various subtypes of the gibberellins (GA1, GA3, and GA4) from *Aspergillus clavatus* strain Y2H0002. Waqas et al. (2014) have recorded the production of various subtypes of GA such as GA1, GA3, GA4, GA7, and GA9 in *Aspergillus fumigatus* and other fungi isolated from the roots of *Glycine max* (L.) Merr and *Cucumis sativus* however, the amount of various GA produced by fungus was less (0.05–10.55 $ngmL^{-1}$). Hamayun et al. (2009) have also reported the production of GA in *A. fumigatus*, however, the amount of GA produced by fungus was in nanogram quantity. Khan et al. (2011) have reported production of GA by endophytic *A. fumigatus* sp LH02 and found that this fungus influences endogenous level of phytohormone and production of isoflavonoid in plants. Yadav et al. (2011) have also reported production of IAA by *Aspergillus niger*. Many researchers have reported the production of phytohormones by *Aspergillus* and other fungi isolated from rhizosphere of various plants (Hamayun et al. 2009, 2017; Khan et al. 2011). Our study also confirmed the production of GA and IAA in *Aspergillus* sp NPF7 isolated from wheat rhizosphere, however, the yield of IAA and GA produced by our isolate was comparatively higher than the yields reported by other groups.

Change in the color of CAS agar and CAS reagent from blue to orange-red is due to the removal of iron from hexadecyl trimethyl ammonium bromide (a weak chelator in CAS reagent) by siderophore (strong Fe chelator) produced by the

fungus. Milagres et al. (1999) and Patel et al. (2018) have also reported a color change of CAS agar and CAS reagent due to siderophore production by different rhizosphere microbes. Renshaw et al. (2002) also reviewed siderophore producing potential of fungi and their applications in plant growth promotion. Haas (2014) have reported siderophore production from *A. fumigatus*. Siderophore production in *A. niger* CSR3 isolated from *Cannabis sativa* has been recorded by Lubna et al. (2018b). Siderophore producing PGPF are more beneficial as their iron solubilizing potential is comparatively higher than the siderophore producing PGPR.

The appearance of the zone of hydrolysis of tri-calcium phosphate on PKA in PKB indicated the P solubilization potential of NPF7. *Aspergillus awamori* S19 isolated from the rhizosphere soil have been reported to solubilize insoluble phosphate (Jain et al. 2011). Similarly in a study by Bhattacharya et al. (2013) a potent fungal strain *Emericella nidulans* isolated from vermicompost exhibited P solubilization potential. Yadav et al. (2011) have also reported P solubilization (328 μgmL^{-1}) by *A. niger* PSF-7. Saxena et al. (2016) recorded solubilization of 354.41 mg of P by *A. awamori*. P solubilization potential in *Aspergillus* sp. has been claimed by various groups, our study also confirms the P solubilization activity but with much higher potential (790 mgL^{-1}) as compared to the P solubilization in *Aspergillus* sp. observed by other groups.

Inoculation of wheat and chickpea seeds with *Aspergillus* sp. NPF7 significantly promoted growth, seed germination, root length, and shoot length parameters vis-a-vis control. Khan et al. (2011) have claimed GA producing endophytic *A. fumigatus* sp. LH02 promotes plant growth and tolerance against salinity stress in rice plant. Lubna et al. (2018b) have reported plant growth promotion and increase in chlorophyll content in rice due to inoculation with MPGP traits producing *A. niger* CSR3. You et al. (2015) have observed plant growth promotion activity of *A. clavatus* strain Y2H0002 in rice seedlings under diverse environmental conditions. Muhammad et al. (2014) also claimed the inoculation of *A. fumigatus* results in a significant increase in growth and yield in rice. Hamayun et al. (2009) have also reported the plant growth promotion activity of *A. fumigatus* in soybean. Increase in shoot length, root length and dry weight of shoot and root in chickpea due to inoculation of *A. niger* have been recorded by Yadav et al. (2011). Growth promotion and increased uptake of P in roots, shoots, and grains of wheat plants following the inoculation with *Aspergillus tubingensis* and *A. niger* has been recorded by Gurdeep Kaur and Reddy (2017). They also claimed an increase in soil fertility due to inoculation of *Aspergillus* sp. Saxena et al. (2016) found plant growth promotion in chickpea due to the synergistic effect of P solubilizing *A. awamori* and plant growth promoting *Pseudomonas fluorescens*.

A local non-aflatoxigenic isolate producing multiple plant growth promoting traits is of agriculture importance for use as multipotent and sustainable bioinoculant as it is well adapted to the local agro-climatic conditions. Production of the copious amount of IAA, GA, siderophores and P solubilization by *Aspergillus* sp. NPF7 is indicative of multiple bioactive potentials of this isolate. The ability of this isolate to promote seed germination, development of shoot and root elongation in wheat and chickpea reflected its bioefficacy as PGPF.

Conclusion

Aspergillus sp. NPF7 obtained from the local wheat rhizosphere of Surat, Gujarat produced multiple plant growth promoting traits like IAA, GA, and siderophore, solubilized phosphate and promoted seed germination, root length and shoot length in wheat and chickpea plants. Such multipotent fungus obtained from local rhizosphere and used in the same conditions is expected to perform better due to its proper adaptation to the local soil environmental conditions and hence can be used as an eco-friendly and natural alternative to hazardous chemical fertilizers leading to sustainable agriculture.

Acknowledgements This research was supported by Gujarat State Biotechnology Mission (GSBTM), FAP-2010. The authors wish to thank GSBTM for financial support.

References

Adachi M, Sako Y, Ishida Y (1996) Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. J Phycol 32:424–432

Albermann S, Elter T, Teubner A, Krischke W, Hirth T, Tudzynski B (2013) Characterization of novel mutants with an altered gibberellin spectrum in comparison to different wild-type strains of *Fusarium fujikuroi*. Appl Microbiol Biotechnol 97:7779–7790

Berryos J, Illanes A, Aroca G (2004) Spectrophotometric method for determining gibberellic acid in fermentation broths. Biotechnol Lett 26:67–70

Bhattacharya SS, Barman S, Ghosh R, Duary RK, Goswami L, Mandal NC (2013) Phosphate solubilizing ability of *Emericella nidulans* strain V1 isolated from vermicompost. Indian J Exp Biol 51:840–848

Brick JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl Environ Microbiol 57:535–538

Cassán F, Vanderleyden J, Spaepen S (2013) Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum*. J Plant Growth Regul. https://doi.org/10.1007/s00344-013-9362-4

Davičre JM, Achard P (2013) Gibberellin signaling in plants. Development 140:1147–1151

Gaur AC (1990) Physiological functions of phosphate solubilizing microorganisms. In: Gaur AC (ed) Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific Publishers, New Delhi, pp 16–72

Gurdeep Kaur M, Reddy S (2017) Improvement of crop yield by phosphate-solubilizing *Aspergillus* species in organic farming. Arch Agro Soil Sci 63(1):24–34. https://doi.org/10.1080/03650340.2016.1182161

Haas H (2014) Fungal siderophore metabolism with a focus on *Aspergillus fumigatus*. Nat Prod Rep 31:1266–1276

Hamayun M, Khan SA, Khan AL, Rehman G, Sohn EY, Shah AA, Kim SK, Joo GJ, Lee IJ (2009) *Phoma herbarium* as a new gibberellin-producing and plant growth-promoting fungus. J Microbiol Biotechnol 19(2):1244–1249

Hamayun M, Anwar H, Sumera AK, Kim HY, Abdul LK, Waqas M, Irshad M, Amjad I, Gauhar R, Samin J, Lee IJ (2017) Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. Front Microbiol 8:686–692

Holbrook AA, Edge WJW, Bailey F (1961) Spectrophotometric method for determination of gibberellic acid. Adv Chem 28:159–167

Jadhav HP, Sayyed RZ (2016) Hydrolytic enzymes of rhizospheric microbes in crop protection. MOJ Cell Sci Rep 3:00070

Jadhav HP, Shaikh SS, Sayyed RZ (2017) Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: an overview. In: Mehnaaz S (ed) Rhizotrophs: plant growth promotion to bioremediation. Springer, Singapore, pp 183–203

Jain R, Saxena J, Sharma V (2011) Solubilization of inorganic phosphates by *Aspergillus awamori* S19 isolated from rhizosphere soil of a semi-arid region. Ann Microbiol 62:725–735

Kang SM, Joo GJ, Hamayun M, Na CI, Shin DH, Kim HY, Hong JK, Lee IJ (2009) Gibberellin production and phosphate solubilization by a newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. Biotechnol Lett 31:277–281

Khan SA, Hamayun M, Yoon H, Kim HY, Suh SJ, Hwang SK, Kim JM, Lee IJ, Choo YS, Yoon UH, Kong WS (2008) Plant growth promotion and *Penicillium citrinum*. BMC Microbiol 8:231

Khan A, Hamayun M, Kim YH, Kang SM, Lee JH, Lee IJ (2011) Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. Process Biochem 46:440–447

Khan AL, Hamayun M, Kang SM, Kim YH, Jung HY, Lee JH, Lee IJ (2012) Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. BMC Microbiol 12:3–9

Khan AL, Hussain J, Al-Harrasi A, Al-Rawahi A, Lee IJ (2013) Endophytic fungi: resource for gibberellins and crop abiotic stress resistance. Crit Rev Biotechnol. https://doi.org/10.3109/07388551.2013.800018

Lubna B, Asaf S, Hamayun M, Gul H, Lee I-J, Hussain A (2018a) *Aspergillus niger* CSR3 regulates plant endogenous hormones and secondary metabolites by producing gibberellins and indole acetic acid. J Plant Interact 13(1):100–111. https://doi.org/10.1080/17429145.2018.1436199

Lubna B, Sajjad A, Muhammad H, Humaira G, Amjad I, Ihsan U, In-Jung L, Anwar H (2018b) Plant growth promoting endophytic fungi *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis. https://doi.org/10.1007/s13199-018-0545-4

Meyer JM, Abdallah MA (1978) The fluorescent pigments of *Pseudomonas fluorescens*: biosynthesis, purification and physicochemical properties. J Gen Microbiol 107:319–328

Milagres AM, Machuca A, Napoleao D (1999) Detection of siderophores production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. J Microbiol Methods 37:1–6

Mineo L (1990) Plant tissue culture techniques; in tested studies in laboratory teachings. Proc ABLE 11:151–174

Murphy J, Riley HP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Parker RE (1979) Continuous distribution: tests of significance. In: Parker RE (ed) Introductory statistics for biology, 2nd edn. Cambridge University Press, London, pp 18–42

Patel PR, Shaikh SS, Sayyed RZ (2016) Dynamism of PGPR in bioremediation and plant growth promotion in the heavy metal contaminated soil. Indian J Exp Biol 54:286–290

Patel PR, Shaikh SS, Sayyed RZ (2018) Modified chrome azurol S method for detection and estimation of siderophores having affinity for metal ions other than iron. Environ Sustain 1:81–87

Payne SM (1994) Detection, isolation and characterization of siderophores. In: Clark VL, Bovil PM (eds) Methods enzymology. Academic Press, New York, pp 329–344

Pereg L, McMillan M (2015) Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems. Soil Biol Biochem 80:349–358

Prashar P, Kapoor N, Sachdeva S (2014) Rhizosphere: its structure, bacterial diversity and significance. Rev Environ Sci Biotechnol 13:63–77

Raed NA, Mohammed HK, Basil AA (2016) Detection of aflatoxigenic producing strains of *Aspergillus flavus* from poultry fees by UV light and ammonia. Basrah J Vet Res 14:169–174

Renshaw JC, Robson GD, Trinci AP, Wiebe MG, Livens FR, Collison D, Taylor RJ (2002) Fungal siderophores: structures, functions and applications. Mycol Res 106:1123–1142

Saito S, Okamoto M, Shinoda S, Kushiro T, Koshiba T, Kamiya Y, Hirai N, Todoroki Y, Sakata K, Nambara E, Mizutani M (2006) A plant growth retardant, uniconazole, is a potent inhibitor of ABA catabolism in Arabidopsis. Biosci Biotechnol Biochem 70:1731–1739

Sambrook J, Russel DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 94–99

Saxena J, Saini A, Kushwaha K, Ariño A (2016) Synergistic effect of plant growth promoting bacterium *Pseudomonas fluorescens* and phosphate solubilizing fungus *Aspergillus awamori* for growth enhancement of chickpea. Indian J Biophys Biochem 53:135–143

Sayyed RZ, Chincholkar SB, Reddy MS, Gangurde NS, Patel PR (2013) Siderophore producing PGPR for crop nutrition and phytopathogen suppression. In: Maheshwari DK (ed) Bacteria in agrobiology: disease management. Springer, Berlin, pp 449–471

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:46–56

Shaikh SS, Patel PR, Patel SS, Nikam SD, Rane TU, Sayyed RZ (2014) Production of biocontrol traits by banana field fluorescent Pseudomonads and comparison with chemical fungicide. Indian J Exp Biol 52:917–920

Sharma SB, Sayyed RZ, Trivedi MH, Thivakaran GA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2:587. https://doi.org/10.1186/2193-1801-2-587

Sharma SB, Sayyed RZ, Sonawane MS, Trivedi MH, Thivakaran GA (2016) *Neurospora* sp SR8, a novel phosphate solubilizer from rhizosphere of soil of Sorghum in Kachh, Gujarat, India. Indian J Exp Biol 54:644–649

Taiwo LB, Ailenokhuoria BV, Oyedele AO (2017) Profiling rhizosphere microbes on the root of maize (*Zea mays*) planted in an alfisol for selection as plant growth promoting Rhizobacteria (PGPR). Microbiol Res J Int 21:1–10

Unyayar S, Topcuoglu SF, Unyayar A (1996) A modified method for extraction and identification of indole-3-acetic acid (IAA), gibberellic acid (GA3), abscisic acid (ABA) and zeatin produced by *Phanerochaete chrysosporium*, ME 446. Bulg J Plant Physiol 22:105–110

Waqas M, Khan AL, Lee I-J (2014) Bioactive chemical constituents produced by endophytes and effects on rice plant growth. J Plant Interact 9(1):478–487. https://doi.org/10.1080/17429145.2013.860562

Yadav J, Verma JP, Tiwari KN (2011) Plant growth promoting activities of fungi and their effect on chickpea plant growth. Asian J Biol Sci 4:291–299

Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annu Rev Plant Biol 59:225–251

You Y-H, Kwak TW, Kang S-M, Lee M-C, Kim J-G (2015) *Aspergillus clavatus* Y2H0002 as a new endophytic fungal strain producing gibberellins isolated from *Nymphoides peltata* in fresh water. Mycobiology 43(1):87–91. https://doi.org/10.5941/MYCO.2015.43.1.87