



In vitro production of IAA by endophytic fungus *Aspergillus awamori* and its growth promoting activities in *Zea mays*

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Abstract

Indole-3-acetic acid (IAA) plays an important role in plant microbe interactions. Current study explored the role of indole-3-acetic acid (IAA) as a signalling molecule for chemical dialogue between endophytic fungus and host plant roots. The endophytic fungus was isolated from the leaves of drought stressed *Withenia somnifera* and was identified as *Aspergillus awamori* W11 through ITS region of 18 S rDNA sequence. The isolated W11 strain was capable of producing important secondary metabolites, including IAA, phenols and sugars. Culture filtrate of the strain contained 24.2, 275.4 and 127.4 µg/mL of IAA, phenols and sugars, respectively. The strain has efficiently colonized the maize roots and enhanced the growth of host plant. In order to determine the role of IAA in root colonization by endophyte, we inhibited the biosynthesis of IAA. Inhibition of IAA production by foliar application of yucasin effectively reduced the colonization of endophyte in maize roots by 52%, whereas root application of yucasin has decreased the colonization frequency by 66%. However, the application of IAA restored the ability of *Aspergillus awamori* to colonize maize roots and significantly improved various growth parameters. From these observations, it is concluded that IAA plays a vital role in initiating a crosstalk between the two partners.

Keywords Fungal endophytes · Yucasin · Plant metabolites · Maize roots

1 Introduction

Endophytes are microorganisms (fungi or bacteria) that are widespread in plant tissues, inhabiting their host asymptotically. They may complete all parts of their life cycle within their host or prefer to spend only part of their life cycle inside host tissues (Gouda et al. 2016). Transmission of endophytic fungi to host species occurs either horizontally via spores or vertically by invading the seeds (Rodriguez et al. 2009). These fungal symbionts can profoundly affect plant ecology, adaptability and evolution (Rodriguez et al. 2009), and are important for community structure and associated biodiversity

(Vila-Aiub et al. 2003). Endophytic fungi provide a number of benefits to the host plants in various ways, such as growth promotion, protection against diseases and pests, and augmenting absorption of minerals (Sieber et al. 2002). Colonization of endophytic fungi enhances the ecological adaptability of the plant by improving its tolerance against the biotic and abiotic stresses (Dastogeer et al. 2017).

The existence of microbes in the rhizosphere is greatly dependent on the root exudates (Badri et al. 2009). Likewise, the endophytes benefit their host by adding valuable secondary metabolites to the endogenous pool of their host and in return get food and shelter (Schulz and Boyle 2005). Indole-3-acetic acid is a type of auxin that has been widely reported in endophytes and believed to be an important tool for plant growth promotion (Spaepen et al. 2007). The role of microbial IAA in plant-microb interactions has received increasing attention in recent years. In addition, some studies showed that IAA can act as a signalling molecule in microorganisms because it affects gene expression in those microorganisms (Yuan et al. 2008). Beneficial plant-microbes, mainly fungi have always been an area of interest because in-depth study of such relationships may lead to sustainable agriculture.

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Roots produce a variety of organic compounds, including sugars, organic acids and vitamins (Fu et al. 2015). These compounds are then used by fungi as nutrients or signals. In contrast, fungi release iron carriers, volatile compounds, and plant hormones that may enhance plant growth, either directly or indirectly, by increasing nutrient availability to their host (Huang et al. 2014). IAA produced by fungi can induce lateral root formation and root hair development (Contreras-Cornejo et al. 2009), thus enhancing nutrient absorption capacity of the plants. The role of fungal produced IAA in different plant-fungal interaction can also lead to changes in the basal defence mechanisms of the plant (Fu et al. 2015). The IAA produced by fungi can defeat pathogenic strains and disease progression by enhancing the plant's immune response (Ludwig-Muller 2015). Similarly, phenolic acids and their derivatives (secondary metabolites that are produced by the plants) plays multifunctional role in rhizosphere-microbial interactions (Martens 2002). Phenolic acids are produced by the plants through phenylpropanoid pathway or as breakdown products of lignin and cell wall polymers in vascular plants (Croteau et al. 2000). Microbes have also shown the ability to produce phenolic acids (Rimmer and Abbott 2011). Phenolic acids and lignin found in plant cell walls have a unique chemical structure, consisting of C6-C3 (phenylpropanoid type), while the microbial-derived phenolic acids are of C6-C1 (benzyl type) (Mandal et al. 2010).

Medicinal plants have shown to be great reservoirs of the endophytic fungi that are believed to be involved in the production of pharmaceutical products (Zhang et al. 2006). Similarly, plants growing in dry areas have higher capacity to accommodate more endophytes. In current study, an important medicinal plant, *Withania somnifera*, from dry area of district Mardan was explored for the existence of potential maize (*Zea mays* L.) growth promoting endophytic fungi. *Withania somnifera* is an erect, evergreen, downy shrub, 30 to 150 cm tall, with thick, fleshy rhizomes. The leaves are simple, ovate and glabrous (Khan et al. 2010). Maize is an essential multipurpose crop in Pakistan and is classified as the third most important cereal after wheat and rice (Rafiq et al. 2010). It is used as human food (especially in the mountains), livestock feed and wet grinding industries. In addition, corn-based products (corn flakes, corn flour, etc.) and their by-products (corn gluten, starch) are more widely used in food and feed companies. Due to its wide availability, its production has increased from 3313 tons in 2008 to 3487 tons in 2010 (Fahad and Bano 2012). The main growing areas are concentrated in two provinces, Punjab and KPK. In addition, it is cultivated twice a year, i.e. fall maize (July / September) and spring maize (February / March). As an important staple food in the world (Khatoun et al. 2012), maize has been

widely studied and it has been found (in cereals) to be a very good substrate for fungal growth and toxigenicity (Khatoun et al. 2012). Keeping the above discussion in view, the present study was aimed to isolate IAA producing endophytic fungi from a medicinal plant *Withania somnifera* to know their role in root colonization and plant growth promotion.

2 Materials and methods

The experiments were conducted in plant microbe interaction laboratory, Abdul Wali Khan University Mardan, Pakistan. Maize variety GAUHER was obtained from National Agriculture Research Center (NARC), Islamabad. The seedlings were allowed to grow for 14 days inside growth chamber (DAIHN Lab Tech) under controlled conditions (photoperiod 16/8 h and temperature 25 °C at midday and 15 °C at night; 390 ppm CO₂; 40% humidity).

2.1 Plant material and isolation of endophytic fungi

Withania somnifera was used for the isolation of bacterial and fungal endophytes by the well-established method of Pandey et al. (2018). Healthy leaves of *Withania somnifera* were collected from drought stress area of district Mardan. The plant materials were brought to the laboratory in sterile polythene bags and were processed within 24 h to diminish the risks of contamination. The collected leaf samples were rinsed with tap water and surface sterilized by dipping it in 5% sodium hypochlorite solution for 5 s. The leaf samples were then dipped into 95% ethanol for 3 min and rinsed (5 times) with autoclave double distilled water. The sterilized leaves were air dried under sterile condition to remove excess moisture. After drying, the leaves were approximately cut into 0.5 cm disks using a flame sterilized scalpel. About 5 to 6 discs were placed on Hegam medium plates (0.5% glucose, 0.05% KH₂PO₄, 0.05% MgSO₄.7H₂O, 0.05% NH₄Cl, 0.1% FeCl₃, 80 ppm streptomycin and 1.5% agar; pH 5.6 ± 0.2) for one week (Hamayun et al. 2010). Imprints of the surface sterilized explants on Hegam medium were used to test the efficiency of surface sterilization protocol (Lubna et al. 2018). For purification, the developing fungal plugs were grown on potato dextrose agar (PDA) medium plates. The culture filtrate and biomass of the purified fungus were then grown in 250 mL flask containing 50 mL czapek broth medium (1% glucose, 1% peptone, 0.05% KCl, 0.05% MgSO₄.7H₂O, and 0.001% FeSO₄.7H₂O; pH 7.3 ± 0.2) for seven days at 28 °C and 120 rpm in shaking incubator (Model No: NB-205 V/NB-205VL) (Khan et al. 2008).

2.2 Determination of colonizing frequency

Colonization frequency (CF %) of the isolated strain was determined as described by Suryanarayanan et al. (2003) and Photita et al. (2001). Colonization frequency was calculated as:

%Colonization frequency

$$= \frac{\text{Total number of root colonized}}{\text{Total number of roots}} \times 100$$

2.3 Screening of isolates for plant growth promoting characters including ammonia and IAA

The isolated strains were screened for the production of ammonia as described by Chadha et al. (2015). Endophytes were grown in 15 mL czapek broth contained in 20 mL test tubes, under previously described culture conditions. After 7 days, culture filtrate (CF) was obtained as mentioned above and 0.5 mL of Nessler's reagent (potassium iodide 50 g/50 mL cold water, mercuric chloride 22 g in 350 mL water and 5 N NaOH 200 mL dilute to 1 Liter) was added to it. Appearance of brown color indicated the presence of ammonia in the CF. The production of IAA was assayed calorimetrically using Salkowski reagent (Khan et al. 2017).

2.4 Molecular characterization of the isolated strain

Fresh mycelium was collected and fungal genomic DNA was extracted using the SolGent Fungus Genomic DNA Extraction Kit (Cat No. SGD64-S120; SolGent Co., Daejeon, Korea) as described by Waqas et al. (2012). The primers ITS1 (5'TCC GTA GGT GAA CCT TGC GG 3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC 3') were used to amplify the ITS region of rDNA (partial sequences of 18 S and 28S rDNA and complete sequences of ITS1, 5.8S rDNA and ITS2. (Zahoor et al. 2017). The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 µL reaction mixture by using a EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2 min, 35 cycles of 95 °C for 1 min, 55 °C, and 72 °C for 1 min each were performed, finishing with a 10-min step at 72 °C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

2.5 Determination of Indole-3-acetic acid in culture filtrate by HPLC

The isolated strain w11 was screened for IAA production by subjecting the CF to HPLC analysis (Khan et al. 2016). The strain was cultured in czapek broth media containing different concentrations of tryptophan (0, 100, 500, 1000 µg/mL) at 28 °C and 120 rpm in shaking incubator for seven days. After seven days, the culture was harvested and filtered through Whatman No. 1 filter paper. A high performance liquid chromatography (HPLC) system equipped with a differential ultraviolet (UV) detector (set at 280 nm) and a C18 column (5 µm; 25 × 0.46 cm) was used to assess the CF for the presence of IAA. The mobile phase consisted of methanol and water (80:20 [v / v]) with a flow rate of 1.5 mL / min. The injection volume was 20 µL. The retention time of the analyte was compared to the retention time of the authentic internal standard. Quantification was done by comparing the peak area (Shahab et al. 2009).

2.6 Effect of yucasin on release of IAA in culture filtrate and biomass production

To check the effect of auxin inhibitor, yucasin [-5-(4-chlorophenyl)-1,2-dihydro-1,2,4-triazole-3-thione], the isolated endophyte was grown in czapek medium containing yucasin (50 µM). After a week, the culture was harvested and sieved through Whatman No. 1 filter paper. Filtrate was then screened for IAA production as described earlier and biomass was collected and kept in 50 mL falcon tube at 60 °C in an oven for 5 days. After five days, the dry biomass was evaluated as described by Klamer and Baath, (2004).

2.7 Maize growth in hydroponic system

To perform root colonization assay, a hydroponic system was setup. The system comprised of 40 mL of half strength Hoagland's solution (Reversat et al. 1999; Barac et al. 2004) in 100 mL beakers with truncated micropipette tips held in sterilized cardboard and sealed with squash tap (Dardanelli et al. 2010). Maize seeds were surface sterilized using HgCl₂ solution (0.1%) and washed three times with distilled water as described above. After surface sterilization, 5 seeds were put into autoclaved petri plates with two fold filter paper and incubated at 28 °C for 5 days and allowed to germinate. The uniform seedlings were transferred to hydroponic set up. After two days of growth, the seedlings were inoculated with endophytic spores' suspension (the inoculum density was adjusted to 10⁶ mL⁻¹ spore with water). Fungal spore suspension was prepared by mixing loopfull spores in distill water. Spores density was determined by adding 10 µL of the spore solution to each side of the hemacytometer and counted the number of spores on both sides of hemacytometer under a light microscope.

2.8 Root colonization assay

To determine fungal colonization in maize roots, plants were collected after 14 days of inoculation. Plant roots were initially washed with tap water and cut into small pieces and stained with lectophenol cotton blue dye (Cotton blue 0.05 g, phenol crystals 20 g, glycerol 40 mL, lactic acid 20 mL, distilled water 20 mL) for 20 min. After 20 min, the segments were washed and observe under light microscope. The level of colonization was quantified by placing the surface sterilized root segments on PDA. The root were surface sterilized with 0.1% HgCl₂ and washed with distill water and cut into 1 cm fragments. Six root segments per plant, i.e. 2 from upper part, 2 from middle part and 2 from lower part were plated on PDA plates for one week. After incubation for a week, colonization percentage was measured by dividing number of root segments positive for colonization by total number of root segments studied and multiplied by 100%. For comparison and validation of surface sterilization procedure, non-injured root segments were plated on PDA. Also, the root exudates of inoculated and non-inoculated seedlings were collected and screened for the determination of IAA.

2.9 Exogenous application of IAA

For the exogenous application of IAA, plants were grown in a 100 mL beaker having half strength Hoagland solution (40 mL). After two days of growth in liquid solution, standard IAA at a final concentration of 10 µg/mL was applied through aerial spray on plants (Vandeleur et al. 2014). The seedlings were then inoculated with fungi as described earlier, a control experiment (uninoculated seedlings) were carried simultaneously. Seedlings were harvested on the 14th day and various growth parameters, such as shoot length, root length, and dry weights were measured and roots exudates were screened for determination of IAA.

2.10 Inhibition of IAA biosynthesis by yucasin

Biosynthesis and exudation of IAA by the root was inhibited by applying yucasin (50 µM) in the form of foliar spray (Nishimura et al. 2014). Root colonization by the endophyte was assayed in the presence of yucasin to assess the role of IAA in root colonization by endophytes.

2.11 Statistical analysis

The data were statistically analyzed by using SPSS for windows 16.0 (SPSS Inc., Chicago, IL, USA). One way ANOVA was applied for the confirmation of the variability and validity of results. Duncan's multiple range test was performed to determine significance between the treatments at $P = 0.05$.

3 Results

3.1 Isolation and preliminary screening of endophytic fungi

A total of four endophytes were isolated from the leaves of *Withenia somnifera*. Strain w12 from *Withenia somnifera* was sprouted from 15 leaves segments and showed highest colonization frequency (65%) among the fungal isolates. The strains w11 and w13 and w14 were least abundant in leaves of *W. somnifera* with 15% colonization frequency. Moreover, only two of the isolated strains (w13 and w11) were able to produce IAA, whereas strain w11 has produced ammonia (Table 1). The production of IAA and ammonia suggested that w11 may be suitable candidate for plant growth promotion and hence was selected for further study.

3.2 Molecular identification of the selected strain w11

Genomic DNA of the fungal isolate w11 was subjected to PCR amplification, using ITS1 and ITS4 primers and the amplified fragment was then sequenced. After aligning the reads from forward and reverse primers, the consensus sequence was subjected to homology analysis using NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic tree was constructed by aligning the ITS sequences of our isolate with corresponding sequence obtained from Gen Bank through NCBI BLAST. The isolate w11 was identified as *Aspergillus awamori* that showed 100% sequence similarity having 100% query coverage with the sequence of *A. awamori* (Fig. 1). The sequence was submitted to the gene bank under accession number MH986194.

3.3 Effect of yucasin and tryptophan on endophyte growth and IAA production

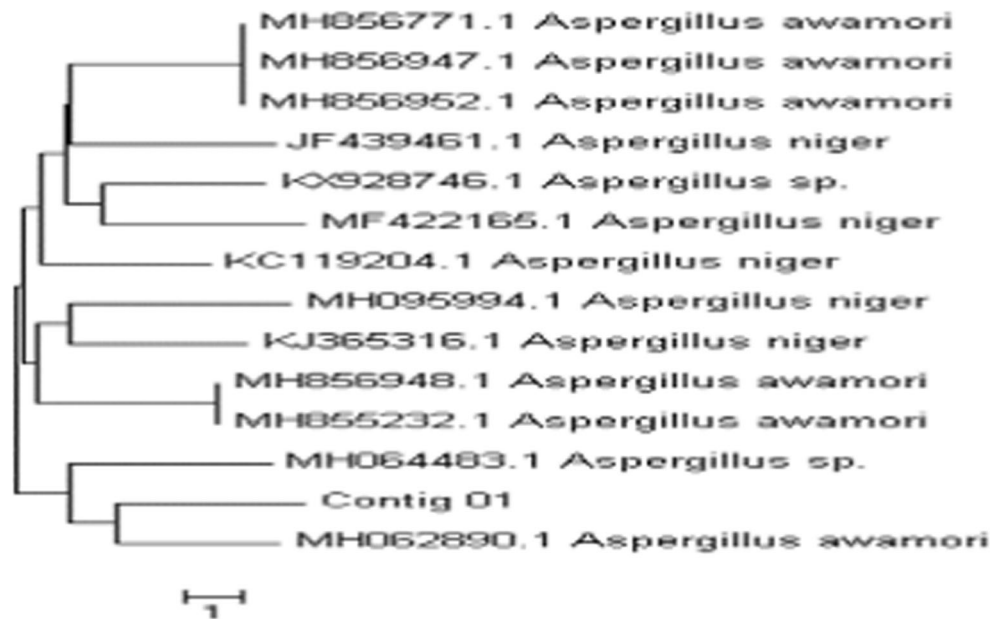
Exposure to yucasin was inhibitory to endophyte growth measured in the form of dry biomass. Yucasin exposed culture had

Table 1 Characterization of endophytic fungi isolated from *W. somnifera* for plant growth promoting characters including IAA and ammonia production along with their colonization frequency

Characters	Fungal Species			
	W11	W12	W13	W14
IAA	+	–	+	–
NH ₃	+	–	–	–
Colonization (%)	15	65	15	25

– Absent, + present

Fig. 1 ITS based phylogenetic analysis of *Aspergillus awamori* w11 by NJ method. Fungal isolate w11 formed a subclade with *Aspergillus awamori*



significantly lower biomass (32%) than the culture with no IAA inhibitor.

Culture filtrate of the strain w11 contained 24.8 $\mu\text{g}/\text{mL}$ of IAA. The production of IAA was found to be tryptophan dependent, as the amount of IAA produced by the endophyte was significantly ($P=0.05$) higher in culture supplemented with tryptophan (500 and 1000 $\mu\text{g}/\text{mL}$). However, addition of 100 $\mu\text{g}/\text{mL}$ of tryptophan did not affect IAA production (Fig. 2). The endophyte grown on media containing 500 and 1000 $\mu\text{g}/\text{mL}$ of tryptophan, produced 27% and 40% greater IAA than the control. Exposure of the endophyte w11 to 50 mM yucasin (IAA biosynthesis inhibitor) significantly ($P=0.05$) inhibited (63%) the production of IAA.

3.4 Effect of fungal endophyte w11 strain in different combination with yucasin and IAA on growth of maize seedling

Endophyte w11 improved shoot length by 32% in comparison to control (Fig. 3a). Root length was also significantly ($P=0.05$) enhanced (31%) as compared to the control (Fig. 4b). Similarly, 33% increase in dry biomass of seedlings was recorded in endophyte w11 associated seedlings (Fig. 3c). Exogenous application of phytohormone IAA significantly ($P=0.05$) improved different growth parameters of maize seedlings (Fig. 3a). Also, exogenously applied IAA synergistically interacted with endophyte w11 and promoted the shoot length. A 9% increase in shoot length was observed in plants grown on media containing

Fig. 2 Effect of yucasin and tryptophan on release of IAA by endophytic fungi *Aspergillus awamori* w11. Data are mean of 9 replicates from 3 independent experiments with standard error bars. Bars (same group) labelled with different letters are significantly different (Duncan test; $p < 0.05$)

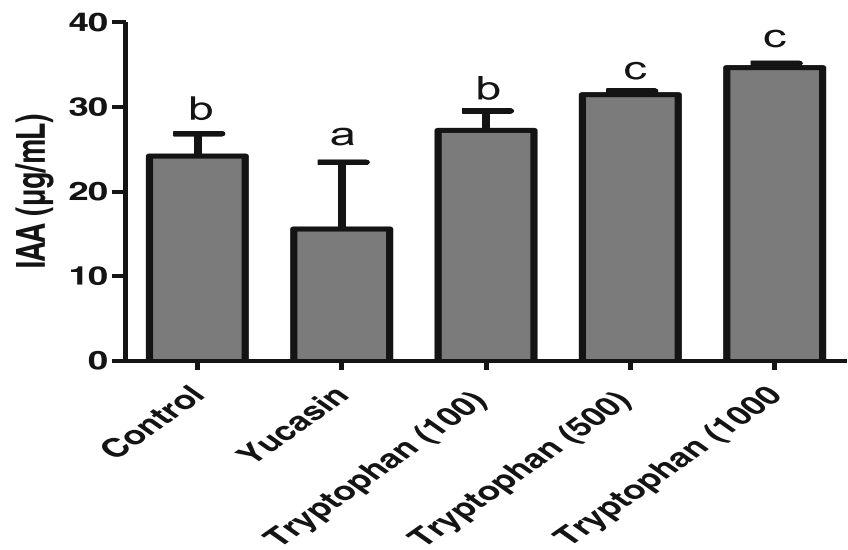
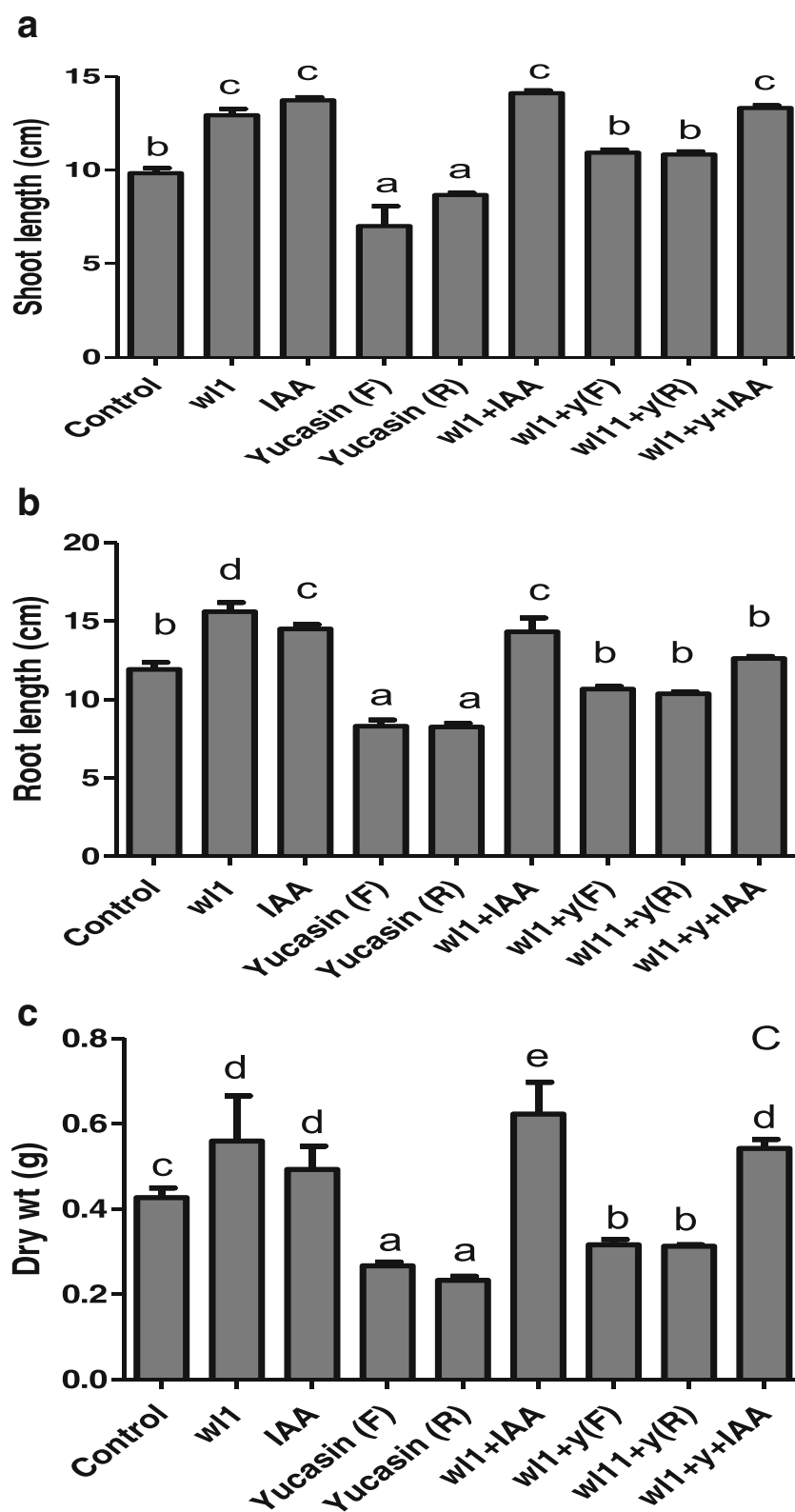


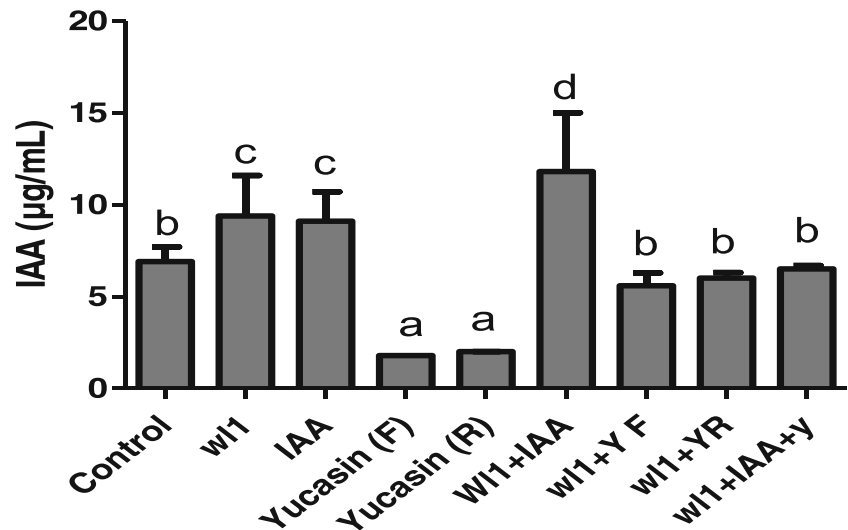
Fig. 3 a-c Effect of endophyte association (w11), IAA (foliar), yucasin (foliar, F and root applied, R) on the **a** shoot length, **b** root length and **c** dry biomass of maize seedlings grown hydroponically in Hogland's solution for 2 weeks. Data are mean of 9 replicates from 3 independent experiments with standard error bars. Bars labelled with different letters are significantly different (Duncan test; $p < 0.05$)



a binary combination of IAA and endophyte. Similarly, exogenous application of IAA in the presence or absence of the endophyte increased seedlings' dry biomass. Application of exogenous yucasin, drastically retarded maize growth. Interestingly,

the endophyte w11 alleviated yucasin stress in maize seedlings restoring their root and shoot growth to the level of control seedlings (Fig. 4a and b). However, growth of endophyte associated maize seedlings exposed to yucasin was significantly

Fig. 4 Effect of IAA and yucasin (foliar, F and root applied, R) on the exudation of IAA by maize roots associated with endophyte *Aspergillus awamori*. Seedlings inoculated with isolated strain and in control were left for growth for 2 weeks. Absorbance was compared with standard curve to find out concentration of IAA in samples. Data are mean of 9 replicates \pm standard error of mean. Similar bars labelled with different letters are significantly different (Duncan multiple range test; $p < 0.05$)



($P = 0.05$) lower than the endophyte associated seedlings without receiving yucasin as an inhibitor. Endophytic association have also enhanced the dry biomass (19%) of the maize seedlings significantly ($P = 0.05$) under yucasin treatment (Fig. 3c). As expected, seedlings' biomass was negatively affected by yucasin (YF) and root applied yucasin (YR), but the presence of w11 has eased the yucasin stress (Fig. 3c).

3.5 Effect of exogenously applied IAA and yucasin on exudation of IAA by endophyte associated maize roots

Highest amount of IAA was found in root exudates collected from endophyte associated maize seedling (9.4 to 11.8 µg/

mL), no matter whether they received exogenous IAA or not. On exposure to yucasin (foliar or root), the seedlings ability to exude IAA was drastically reduced in comparison to the control (Fig. 5a). In yucasin treated seedling, endophyte w11 helped the host to restore the normal secretion of IAA (Fig. 4a).

3.6 Role of IAA in maize root colonization by endophyte w11

The endophyte w11 colonized the maize roots under hydroponic system in all tested treatments (Fig. 5 and Fig. 6). Additionally, colonization frequency of w11 in maize root varied among different root zones. Root zone of division attracted

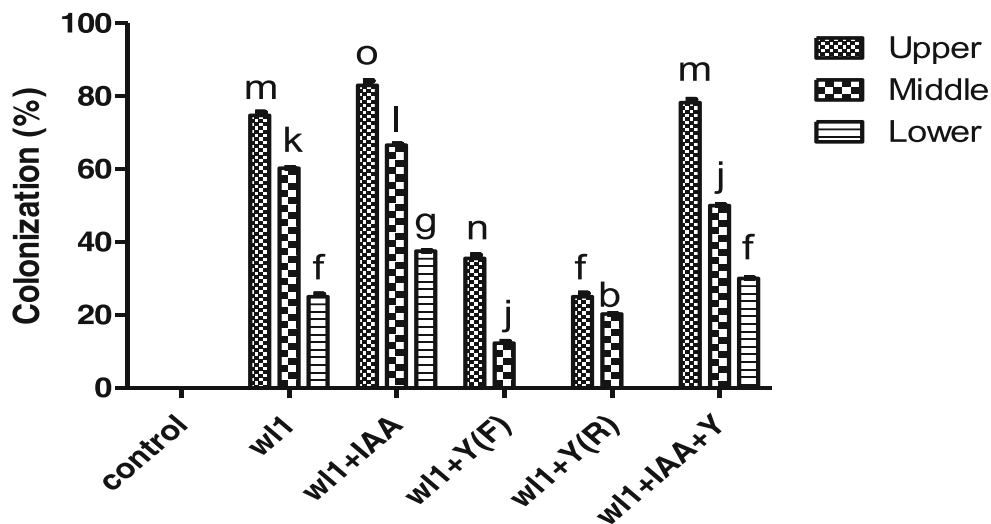


Fig. 5 Effect of yucasin (Y) and IAA on the mean percent colonization of maize roots by endophyte *A. awamori* w11. The inhibitor were applied in the form of foliar spray (F) or in the culture media (R). Other treatments included exogenously applied IAA and IAA+ yucasin. The level of colonization was quantified by plotting the root segments on PDA after 14 days of co-culturing. Six root bricks for each plant, 2 from superior

part (near to inoculum) two from middle part and two from inferior part (near to stem) were kept on clean filter paper and inoculated on PDA plates and incubated for one week. After a week colonization percentage was recorded according to the expression. Percent colonization of endophytic fungi = Number of positive segments/ total number of root segments studied*100

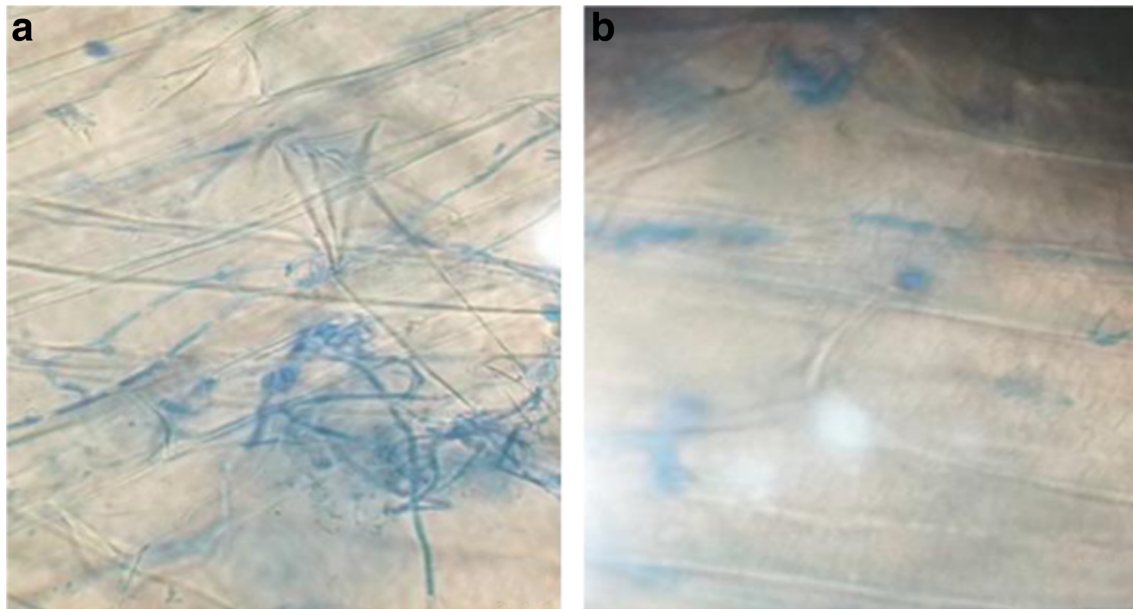


Fig. 6 Visualization of w11 colonization (blue stained hyphae with cotton blue dye) in the maize root **a** endophyte **b** endophyte + yucasin

the endophyte with highest efficiency showing a colonization frequency of 75%. However, colonization frequency dropped in the root above this zone. Similarly, zone of elongation had a colonization frequency of 60%. Area of root in the proximity of stem, i.e. zone of maturation was least colonized (25% colonization frequency). Application of IAA has pronounced effect on root colonization by the w11. IAA has enhanced colonization frequency to 83% in the zone of cell division, 66% in the zone of elongation and 37% in the zone of maturation. Application of yucasin (IAA inhibitor) has negatively influenced the root colonization of w11 strain, reducing its colonization frequency to 52% (foliar application) and 66% (root application), respective.

4 Discussion

In the current study, an endophytic fungus *Aspergillus awamori* w11 was isolated from *Withenia somnifera* leaves and identified by comparative homology of ITS region near the 18 S rRNA gene (Arenal et al. 2007). In fact, fungal endophytes play vital role in host plant growth and yield bioactive metabolites that promote plant-endophyte interaction (Strobel 2003; Hassan et al. 2013). Several bioactive compounds have been isolated from fungi that can act as antifungal and antibacterial (Suryanaryanan et al. 2009). Likewise, endophytic fungi promote plant growth by producing various secondary metabolites, including ammonia and plant hormones, particularly IAA (Fouda et al. 2015). The above mentioned secondary metabolites (IAA, phenols and sugars) have also been found in the culture filtrate of the isolated endophyte *A. awamori* w11. The endophyte w11 released significant

quantity of IAA (24.2 $\mu\text{g}/\text{mL}$) in their culture filtrate, which was comparatively higher than the previously reported endophyte by Waqas et al. (2014). Production of this phytohormone is common among plant growth promoting endophytes and bacterial pathogens. For instance, bacterial pathogens, including *Pseudomonas* and *Xanthomonas* are known to synthesize IAA (Khan et al. 2018). However, there are reports that IAA production is an important tool of biocontrol against fungal pathogens, such as *Colletotrichum* spp. (Yue et al. 2000). Beside this, exogenous IAA has been proved to improve plant growth under normal and stressed conditions (Kaya et al. 2013). Both microorganism and plants have the ability to produce auxin that modulates plant growth and development. IAA is the main auxin that controls all the physiological processes including cell division, tissue differentiation, and responses to light and gravity (Taiz and Zeiger 1998). The biosynthesis of auxin is very complicated; it is likely that several pathways are responsible for the de novo production of auxin. IAA can also be released from IAA-conjugates (IAA-amino acids, IAA-sugar and IAA-methyl ester) by hydrolytic cleavage (Zhao, 2010). In our isolate, IAA production was significantly enhanced by the presence of tryptophan in the media. Tryptophan is considered as a precursor for IAA biosynthesis and its addition to culture medium enhances IAA production (Ahmad et al. 2005). According to our result increasing tryptophan concentration from 500 and 1000 $\mu\text{g}/\text{mL}$ significantly enhanced the amount of IAA by 27% and 40% as compared to the control. Among fungi, the IPA (indole-3-pyruvic acid), pathway is the most common pathway for IAA biosynthesis (Hilbert 2012). TAM (tryptamine pathway) and IAM (indole-3-acetamide) pathways have also been observed in *Ustilago* and *Colletotrichum* sp.

(Hilbert 2012). Quite recently, tryptophan-independent IAA biosynthetic pathways have been discovered in yeast (Rao et al. 2010). Yucasin application on the other hand has remarkably reduced the IAA production in our isolates, which indicated the presence of IPA pathway. Indeed, yucasin inhibited the growth of the endophyte w11 and thus decreased the IAA biosynthesis by the said strain.

In addition to nutrient supply, microorganisms improve plant growth by producing plant hormones (Costacurta and Vanderleyden 1995). The isolated strain was able to colonize maize roots and significantly enhanced shoot and root length and seedling dry biomass ($P > 0.05$). Increase in shoot and root length in fungal inoculated plants have been reported previously by many authors (Hamayun et al. 2010; Meletiadis et al. 2001; Schubert et al. 2009). In current study, *A. awamori* has produced higher amounts of IAA that affected maize growth. Auxins produced by rhizosphere bacteria (Bashan et al. 2004; Khalid et al. 2004), filamentous fungi (Frankenberger and Poth 1987) and yeasts (El-Tarabily 2004) have been described to improve growth and increase yields of host plants. The application of exogenous IAA has significantly increased the shoot length, seedlings' dry biomass and root colonization by isolated strain. Additionally, the efficiency of root colonization by the isolated strain was higher in part of root near to inoculum and sprayed with IAA. Maize seedlings that received exogenous IAA in the form of aerial spray, released greater amount of IAA in root exudates and had greater affinity for *A. awamori* w11. Previously, it was shown that exogenous application of IAA restored in vitro growth as well as efficiency of colonization in rice roots by mutant *Nostoc* spp. (Hussain et al. 2015). To verify the role of IAA in root-endophyte interaction, IAA biosynthesis was inhibited in plant by foliar application of yucasin. Again, colonization was reduced significantly ($P = 0.05$), which indicated that IAA is involved in the establishment of plant-endophyte association. Seedlings exposed to yucasin, released significantly ($P = 0.05$) lower amounts of IAA, confirming the inhibition of its biosynthesis. Interestingly, low IAA exuding maize roots were least colonized by the endophyte pointing the role of this phytohormone in plant-endophyte interaction. Exogenously applied yucasin retarded maize growth. However, yucasin in combination with exogenously applied IAA and endophyte restored seedlings' growth and production of endogenous IAA. According to Hutsch et al. (2002), the total root exudates released by plants into rhizosphere consist of 50–70% of sugars and 10–20% amino acids. Weert et al. (2002) demonstrated that the released amino acid and sugars in rhizosphere doesn't have direct role in nutrients mobilization, but they can attract beneficial microorganisms to support their growth. Seedling associated with endophytic *A. awamori* in binary combination with IAA improved exudation of sugars that might be to attract more plant growth promoting endophytes. A report have clearly shown that plant

can attract and shape the choice of microbes by releasing particular compounds through root exudation, which determine the rhizosphere microbial community (Chaparro et al. 2012).

5 Conclusion

The endophyte *Aspergillus awamori* w11 promote growth of maize seedlings under hydroponic condition. Its ability to produce IAA is responsible for colonizing maize roots and establishing beneficial endophytic associations. Inhibition of IAA limits their ability to interact and carry out symbiotic associations.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest. The work presented is according to current law of the country where carried out.

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