Microbiological Research 207 (2018) 100–107

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Modulation in phenolic root exudate profile of *Abelmoschus esculentus* expressing activation of defense pathway

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ARTICLE INFO

Keywords:
Root exudates
Abelmoschus esculentus
Alcaligenes faecalis
Sclerotium rolfsii
Phenolics
Endophytic bacteria

ABSTRACT

Phenolics play a key role in communication between plants and microbes in the rhizosphere. In this study, shikimic, gallic, fumaric, ferulic, vanillic acid and quercetin in root exudates of *Abelmoschus esculentus* act as chemoattractants of endophytic *Alcaligenes faecalis* strains, BHU 12, BHU 16 and BHU M7. *In vitro* chemotaxis assay showed that BHU 12 expressed highest chemotactic movement (CFU $\sim 50 \times 10^{12}$) towards *A.esculentus* root exudates followed by BHU 16 and BHU M7 (CFU$\sim 9 \times 10^{12}$), thereby confirming their ability to colonize the host rhizoplane region. However, BHU 16 expressed highest biofilm formation ability followed by BHU 12 and BHU M7. Assessment of chemotactic and biofilm formation potential towards individual phenolic acids revealed BHU 12 to be maximally attracted towards 1 µM shikimic acid (2×10^{15}) while BHU 16 towards 1 mM vanillic acid (6.5×10^{12}) and BHU M7 towards 1 mM ferulic acid (3.5×10^{12}), thereby confirming the phenolic acid components responsible for particularly attracting the endophytic isolates. Upon colonization, the endophytic isolates modified the phenolic profiles of root exudates *in planta* in a manner so as to plausibly attract more of the beneficial rhizospheric microbiota as well as self-fortification against pathogenic microbes. This hypothesis was verified by monitoring the changes in phenolic components of *A. esculentus* root exudate owing to *S. rolfsii* infection, a disastrous soil-borne pathogen. Thus, on the whole, the work provides intricate details of plant-endophyte interactions for biotic stress management through careful manipulation of root exudates, thereby aiding in sustainable agriculture.

1. Introduction

The rhizosphere region represents a dynamic ecosystem with the diverse flora, fauna and microbes continuously interacting with each other in a variety of complex reactions (Whipps, 2001; Singh et al., 2016a). Governed by root exudates, these interactions primarily mediate promotion of plant performance in terms of growth and defense (Dakora and Phillips, 2002; Bais et al., 2004, 2006; Badri and Vivanco, 2009; Bertin et al., 2003; Sun et al., 2012; Haichar et al., 2014). Plant root exudates contain not only ions, free oxygen, water, enzymes, mucilage, carbon-containing primary and secondary metabolites, but also phenolics, which selectively stimulate growth of rhizospheric soil microbiota by generating redox reactions in soils thereby altering the composition of microbial communities in different root parts (Northup et al., 1998; Hättenschwiler and Vitousek, 2000; Verbon and Liberman, 2016). Moreover, phenolics subjected to abiotic and biotic reactions contribute to mineralization of soil phosphorus and nitrogen as well as humification (Kefeli et al., 2003; Halvorson et al., 2009). Phenolics are also reported to chelate metals and improve soil porosity leading to an increase in the mobility and bioavailability of essential elements, such as magnesium, potassium, calcium, zinc, copper, manganese, iron, boron and molybdenum, for plant roots (Cesco et al., 2012; Pii et al., 2015).

The phenomenon of chemotaxis and biofilm formation serve as the basic prerequisites for effective bacterial colonization (Sood, 2003; Timmusk et al., 2005; Li et al., 2013; Kimani et al., 2016).Most of the phenolics of root exudates serve as chemotactic signals for a number of soil microorganisms that recognize them and move towards plant roots in the carbon-rich environment of rhizosphere (Perret et al., 2000; Taylor and Grotewold, 2005). In line with the above context, Singh et al. (2016b) reported enhanced chemoattraction of *Bacillus subtilis* towards rice plant due to rutin, a bioflavanoid, which further stimulated antioxidant pathway of the host. The signal system of plants plays an effective role as it screens the approaching microbes by differentially

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https://doi.org/10.1016/j.micres.2017.11.011
Received 15 July 2017; Received in revised form 7 November 2017; Accepted 18 November 2017
Available online 26 November 2017
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stimulating the growth of beneficial ones through the process of root exudation and simultaneously inducing defense mechanisms against pathogens to prevent parasitic interactions. Thus it may be hypothesized that plant species acquire resistance against potential soil-borne pathogens by releasing allelochemicals as root exudates (Dixon, 2001; D'Auria and Gershenzon, 2005; Baetz and Martinoia, 2014). Several reports indicate defensive attributes of root exudates, for instance, *Ocimum basilicum* challenged with *Pythium ultimum* released rosmarinic acid, a caffeic acid derivative with antimicrobial property (Bais et al., 2002). Similarly, Bais et al. (2005) clarified root exudate mediated resistance offered by *Arabidopsis thaliana* to *P. syringae* pathovars. Baetz and Martinoia (2014) suggested that alterations in root exudate components drastically modify the rhizospheric microbiota. In line, Verbon and Liberman (2016) advocated that root exudates influence quorum sensing in soil bacteria while the rhizospheric microbes, in turn modify root exudate composition and induce systemic resistance within host plants.

Abelmoschus esculentus (okra) is a multipurpose crop owing to the various uses of its tissues (Mihretu et al., 2014). While, the fruit serves as a culinary delicacy (Ndunguru and Rajabu, 2004), the mucilage can be utilized as a renewable source of biodegradable material, due to its plasticity, high water solubility, viscosity and elasticity (BeMiller et al., 1993). For these reasons *A. esculentus* is one of the most sought after vegetable crops with India ranking first in its production and export (NCPAH, 2016). However, the yield can be negatively affected by several forms of biotic stresses, such as the attack by fungal phytopathogens, for instance, *Sclerotium rolfsii*, which requires warm and humid temperature conditions for its activity.

Plant inoculation with endophytic microbes protects the host plant from several forms of environmental stresses (Ray et al., 2015, 2016; Mishra et al., 2015). *Alcaligenes faecalis* is well known for its various plant growth promotional attributes, such as, IAA production, phosphate solubilization and ammonia production which eminently aids in root growth as well as antagonistic activity against common soil borne phytopathogens owing to production of hydrogen cyanide and proteolytic activity. These attributes support the candidature of *A. faecalis* as a suitable plant growth promoting and biocontrol agent (Ray et al., 2016; Ndeddy Aka and Babalola, 2016).

This study aims to identify the root exudate phenolics responsible for chemotactically attracting endophytic *A. faecalis* when *A. esculentus* plants were grown under hydroponic conditions. The changes in the composition of root exudate phenolics due to the presence of endophytic microbes were analyzed by high performance liquid chromatography (HPLC). Changes were also observed in root exudate composition in case of okra infected with *S. rolfsii* which enabled the evaluation of basic defense acquired by the host. Thus, this study provides a novel description of sequential changes occurring in phenolic acid compositions of root exudates due to priming by beneficial microbes followed by challenge with harmful phytopathogens.

2. Materials and methods

2.1. Okra root exudates preparation

Seeds of *A. esculentus* (cv. Ujjwal) were surface sterilized using 5% sodium hypochlorite for 10 min followed by three successive rinses with sterile distilled water. The surface sterilized seeds were transferred to 0.5% water agar plates and incubated at 28 ± 2 °C for 2–3 days until emergence of the radicle. For each extraction, 30 seedlings with approximately 2 cm radicle length were transferred to a 50 ml conical flask containing 5 ml of sterile chemotaxis buffer (10 mM potassium phosphate, 0.1 mM ethylene diamine tetra acetic acid(EDTA), 1 mM magnesium sulphate; pH 7.0) and incubated at 28 ± 2 ° C for 24 h. The root exudates released in the buffer were collected and sterilized by passing through a 0.22 µm filter and stored at −80 °C (Yao and Allen, 2006). Total protein concentration of root exudates was determined according to Bradford assay (Bradford, 1976).

2.2. HPLC analyses of A. esculentus root exudates

The collected root exudates were fractionated with ethyl acetate (1:1). The organic fraction was collected and refractionated three times with ethyl acetate to completely collect the organic components of root exudates. The ethyl acetate solution was evaporated and the resulting phenolic components were dissolved in HPLC grade methanol and filtered through 0.22 µm millipore filter.

Analysis of phenolic acids in the exudate was performed according to Singh et al. (2014). The pure phenolic acid standards used for HPLC were purchased from Sigma-Aldrich (St. Louis, MO, USA). Injection volume of the sample was 20 µl. The HPLC, Shimadzu LC-10A (Japan) was equipped with dual pump LC-10A binary system, UV detector SPD-10A, Phenomenex (Torrance, USA) and C18 column (RP-Hydro, 4 µm, 250 mm × 4.6 mm). The mobile phase included 1% acetic acid (A) and acetonitrile (B) with the following gradient elutions: 0 min 82% A plus 18% B at the flow rate of 1 ml min^{-1} for 15 min; 68% A plus 32% B at the flow rate of 1 ml min^{-1} for 25 min; 50% A plus 50% B at the flow rate of 1 ml min^{-1} for 30 min. After 30 min, the run was stopped and optical density of the eluents was detected at 254 nm wherein the peaks of samples were compared with their standard peaks.

2.3. Chemotaxis and biofilm formation by the endophytic bacterial isolates in presence of A. esculentus root exudates

2.3.1. Chemotaxis assay

Qualitative analysis of chemotactic response of the endophytic isolates, BHU 12, BHU 16 (isolated from *A. esculentus* leaf) and BHU M7 (isolated from *Andrographis paniculata* leaf) towards each of the HPLC identified phenolic components of okra root exudates was performed according to Singh et al. (2016b). 24 h old bacterial cultures (10 µl) were spot inoculated on swarm agar plates comprising of 0.8% nutrient broth, 0.5% glucose and 0.4% agar. Each of the HPLC identified phenolic acids at three different concentrations, i.e. 1 mM, 1 µM and 1 nM were incorporated into the plates in form wetted filter paper discs. The plates were further incubated at 28 ± 2 °C for 10 h for assessing chemotactic motility towards the different phenolic acids.

Quantitative assessment of chemotactic ability of BHU 12, BHU 16 and BHU M7 towards each of the HPLC identified phenolic acids was evaluated according to Tan et al. (2013) with slight modifications. A 4-cm 25-gauge needle attached to a 2 ml syringe was used to contain 100 µl of crude root exudate and their individual phenolic acids at concentrations of 1 mM, 1 µM and 1 nM, respectively in different experimental setups. The needle was pricked and inserted into an eppendorf tube (200 µl) containing 100 µl of bacterial suspension of each of the strains and the entire setup was incubated in dark under sterilized conditions at 28 ± 2 °C. Bacterial chemotaxis towards crude root exudates was assessed after 2, 4 6, 8, 10 and 12 h, whereas that towards each phenolic acid was evaluated after 1 h.

After the required time interval, the syringe content was transferred to an eppendorf tube containing 1 ml of sterile normal saline and serially diluted. The individual dilutions were plated on nutrient agar plates, incubated at 28 ± 2 °C and observed after 24 h for appearance of bacterial colonies.

2.3.2. Biofilm formation assay

In vitro biofilm formation by BHU 12, BHU 16 and BHU M7 on solid surface in presence of okra root exudates and its phenolic acids was assessed according to Ray et al. (2015). The strains were grown upto mid-log phase in nutrient broth (NB) medium (HiMedia). Aliquots from the respective cultures were inoculated in NB diluted with 1/10 vols of okra root exudates and the individual HPLC identified phenolic acids at concentrations of 1 mM, 1 µM and 1 nM, respectively. 100 µl of the mixture were added to each well of a 96 well PVC microtitre plate. The

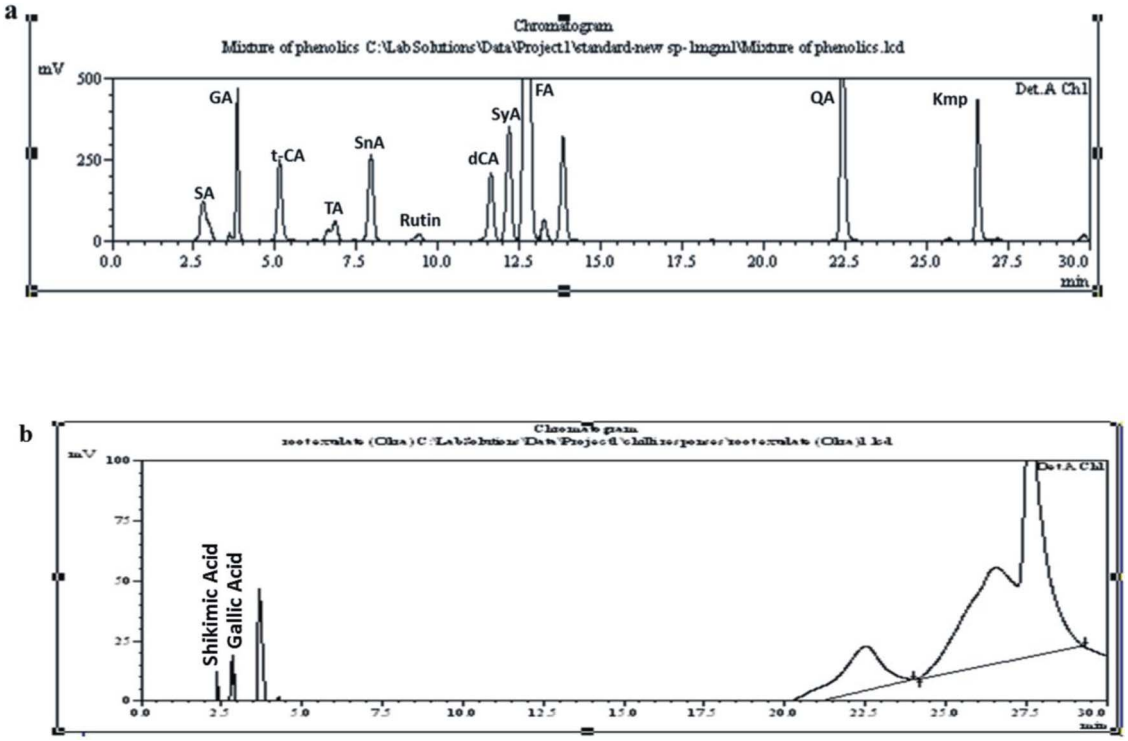

Fig. 1. a) HPLC chromatogram depicting phenolic acid standards (SA: shikimic acid; GA: gallic acid; t-CA: trans cinnamic acid; TA: tannic acid; SA: syringic acid; dCA: 3,5 dihydroxy cinnamic acid; SyA: synaptic acid; FA: ferulic acid; QA: quercetin; Kmp: kaempferol. b) HPLC chromatogram depicting presence of phenolics in root exudates of uninoculated okra plants.

plate was sealed with parafilm and incubated at 28 ± 2 °C for 48 h. After 48 h, the plate was inverted to remove the contents followed by staining of the biofilm so formed with 1% crystal violet (for 10 min) and destaining with 95% ethanol. The amount of biofilm formed was determined by recording the absorbance of bound crystal violet in ethanol at A_{590}.

2.3.3. Inocula preparation, endophytic establishment and pathogen infection

For preparation of inoculum, one ml of log phase culture of each endophytic bacterial isolate was inoculated in 250 ml conical flasks containing 100 ml sterilized NB medium. The suspension was incubated for 48 h at 28 ± 2 °C in a shaker incubator to reach the early stationary phase (CFU ~4×10^8). The cells were harvested by centrifugation at 10,000 rpm for 10 min and the resulting pellets were suspended in 0.85% normal saline and 1% carboxymethyl cellulose (CMC) sodium salt (Merck) to obtain a final CFU count of 4×10^8 (OD ~ 0.4) (Jain et al., 2013).

A. esculentus seeds were surface sterilized with 3% sodium hypochlorite for 10 min and washed with sterilized distilled water. The sterilized seeds were placed on 0.5% water agar and incubated at 28 ± 2 °C for 2–3 days to obtain uniform germination. The germinated seedlings were treated with the bacterial inocula and incubated for 3 h at 28 ± 2 °C. The control consisted of seedlings dipped in sterilized water and CMC and incubated as mentioned above. The seedlings treated with endophytic bacterial isolates and the untreated control seedlings were placed in culture tubes containing sterilized sand and incubated in growth chamber at 30 °C, 76% relative humidity (RH), with a 16:8 h photoperiod.

2.3.4. Root exudate collection and HPLC analyses of endophytic bacteria inoculated A. esculentus plants before and after infection with S. rolfsii

Root exudates were collected from the endophyte inoculated plants after 14 days, prior to infection of plant by *S. rolfsii* according to Yuan et al. (2015). The seedlings were carefully uprooted and the sand was washed with ethyl acetate at room temperature. The organic layer was collected and evaporated to dryness followed by dissolution of the residue in 1 ml HPLC grade methanol. Further extraction and determination of phenolic acids in endophytic bacteria treated host plants and untreated control was proceeded by HPLC as described above.

Two weeks old *A. esculentus* plants were further infected with the pathogen *S. rolfsii* as described by Shokes et al. (1996). A 1 cm^2 mycelial plug of *S. rolfsii* was placed at the collar region of the host plant after slightly removing the rhizospheric sand particles followed by covering the plug with moistened cotton wool to provide the required moisture content. Infected plants without endophytic bacterial treatment served as positive control while untreated uninfected plants served as negative control. The root exudates were collected after 72 h so as to have a substantial amount of exudate.

Extraction and HPLC analyses of phenolic acids were done as described above.

2.4. Statistical analyses

The data were presented as means of three replicates and were subjected to ANOVA. The statistical analyses were done using SPSS 17.0 software. Duncan's test was carried out to analyze the effect of the various treatments.

3. Results

3.1. Analysis of A. esculentus root exudate phenolics and differential migration of the endophytic bacterial strains towards the different phenolic components

HPLC analysis of the root exudates from one week old *A. esculentus* seedlings exhibited the presence of the following phenolic acids, *viz:* shikimic acid (0.111 mg/ml), gallic acid (1.6 mg/ml), vanillic acid (0.04 mg/ml), ferulic acid (0.03 mg/ml), quercetin (0.001 mg/ml) and kaempferol (0.035 mg/ml) (Fig. 1).

The endophytic isolates BHU 12, BHU 16 and BHU M7 displayed an increased motility towards *A. esculentus* root exudates with BHU 12

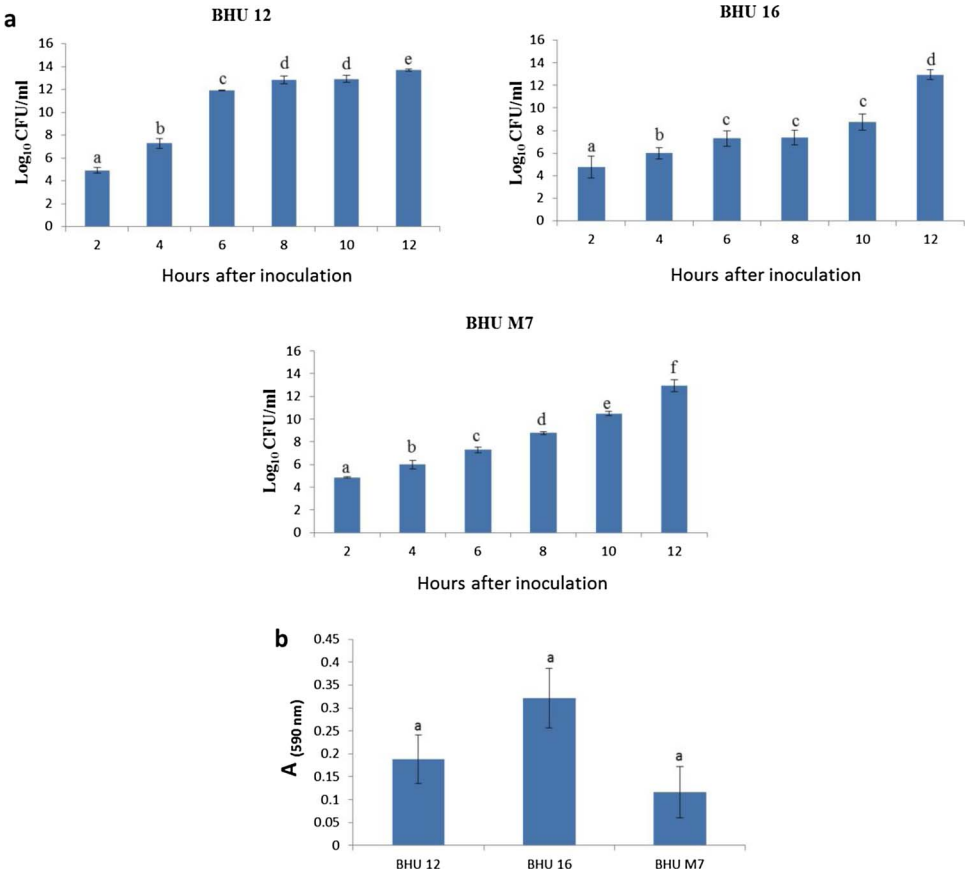

Fig. 2. Chemotaxis and biofilm formation of the endophytic isolates in presence of okra root exudates.

exhibiting highest chemotactic attraction towards *A. esculentus* root exudates (Fig. 2). Surprisingly, BHU M7, a medicinal plant-borne endophytic isolate also expressed distinct chemotactic ability towards the root exudates of host plant (CFU ~ 9×10^{12}).

Biofilm formation ability, an essential prerequisite for successful rhizoplane colonization was also determined in presence of host root exudates, with BHU 16 strain expressing maximum biofilm formation potential followed by BHU 12 and BHU M7. Assessment of migrational ability of the strains towards each of the phenolic acids exhibited BHU 12 as having highest migrational and colonizational potential in presence of shikimic acid with maximum chemotactic movement occurring at 1 µM. Similarly, BHU 16 exhibited highest chemotactic movement towards vanilic acid (1 mM) and BHU M7 towards ferulic acid (1 mM). Also, among the three strains, BHU 12 expressed the highest rate of migration and colonization followed by BHU 16 and BHU M7 (Fig. 3).

3.2. Assessment of phenolic profiles of root exudate of endophyte inoculated hosts

The HPLC chromatogram of *A. esculentus* root exudates after 14 days of treatment with the endophytic isolates expressed significant variation in phenolic acid components. Shikimic and gallic acid concentrations were particularly enhanced in BHU 12, BHU 16 and BHU M7 treated hosts. While BHU 12 treated hosts showed shikimic acid concentration of 1.85 µg g^{-1} fresh weight (FW), BHU 16 and BHU M7 recorded shikimic acid concentrations of 0.83 and 1.9 µg g^{-1} FW, respectively. On the other hand, gallic acid concentrations were 0.17, 0.04 and 0.07 µg g^{-1} FW in BHU 12, BHU 16 and BHU M7 treated hosts, respectively (Fig. 4).

Root exudates released after inoculation with BHU 12 also expressed amounts of epicatechin (1.4 µg g^{-1}), rutin (0.04 µg g^{-1}), ferulic acid (0.004 µg g^{-1}), salicylic acid (0.08 µg g^{-1}) quercetin (0.003 µg g^{-1}) and kaempferol (0.09 µg g^{-1}). Similarly, root exudates of seedlings inoculated with BHU 16 expressed presence of syringic acid (0.02 µg g^{-1}) apart from shikimic and gallic acid as mentioned above. Inoculation with BHU M7 showed the presence of tannic acid, *p*-coumaric acid and daidzein.

3.3. Assessment of phenolic profiles of endophytic inoculated hosts infected with S. rolfsii

Infection of BHU 12 inoculated seedlings with *S. rolfsii* showed the presence of catechin (0.041 µg g^{-1}), shikimic acid (0.544 µg g^{-1}), gallic acid (0.04 µg g^{-1}), tannic acid (0.055 µg g^{-1}), syringic acid (0.37 µg g^{-1}) and ferulic acid (0.018 µg g^{-1}) in root exudates. Similarly, inoculation of infected seedlings with BHU 16 released catechin (0.043 µg g^{-1}), shikimic acid (0.12 µg g^{-1}), gallic acid (0.031 µg g^{-1}) gentisic acid (0.110 µg g^{-1}), ferulic acid (0.005 µg g^{-1}), daidzein (0.0004 µg g^{-1}) and quercetin (0.0009 µg g^{-1}). Likewise, root exudates of infected seedlings inoculated with BHU M7 showed the presence of shikimic acid (0.05 µg g^{-1}), gallic acid (0.02 µg g^{-1}), syringic acid (0.340 µg g^{-1}), vanilic acid (0.03 µg g^{-1}) and *p*-coumaric acid (0.002 µg g^{-1}). In contrast, the infected control plants, released only shikimic (0.055 µg g^{-1}), and gallic acid (0.031 µg g^{-1}). Thus, concentrations of shikimic and gallic acid in root exudates of endophyte inoculated and pathogen infected plants were 8.9 and 0.33 folds higher, respectively for BHU 12, while 1.18 and 0.03 folds higher, respectively for BHU 16 compared to those of the infected control. However, a 0.33 fold decline in gallic acid content was observed in seedlings inoculated with BHU M7 (Fig. 5).

4. Discussion

In the current study, the three endophytic strains, BHU 12, BHU 16 and BHU M7 exhibited significant chemotaxis and biofilm formation in presence of *A. esculentus* root exudates. This suggests that the strains

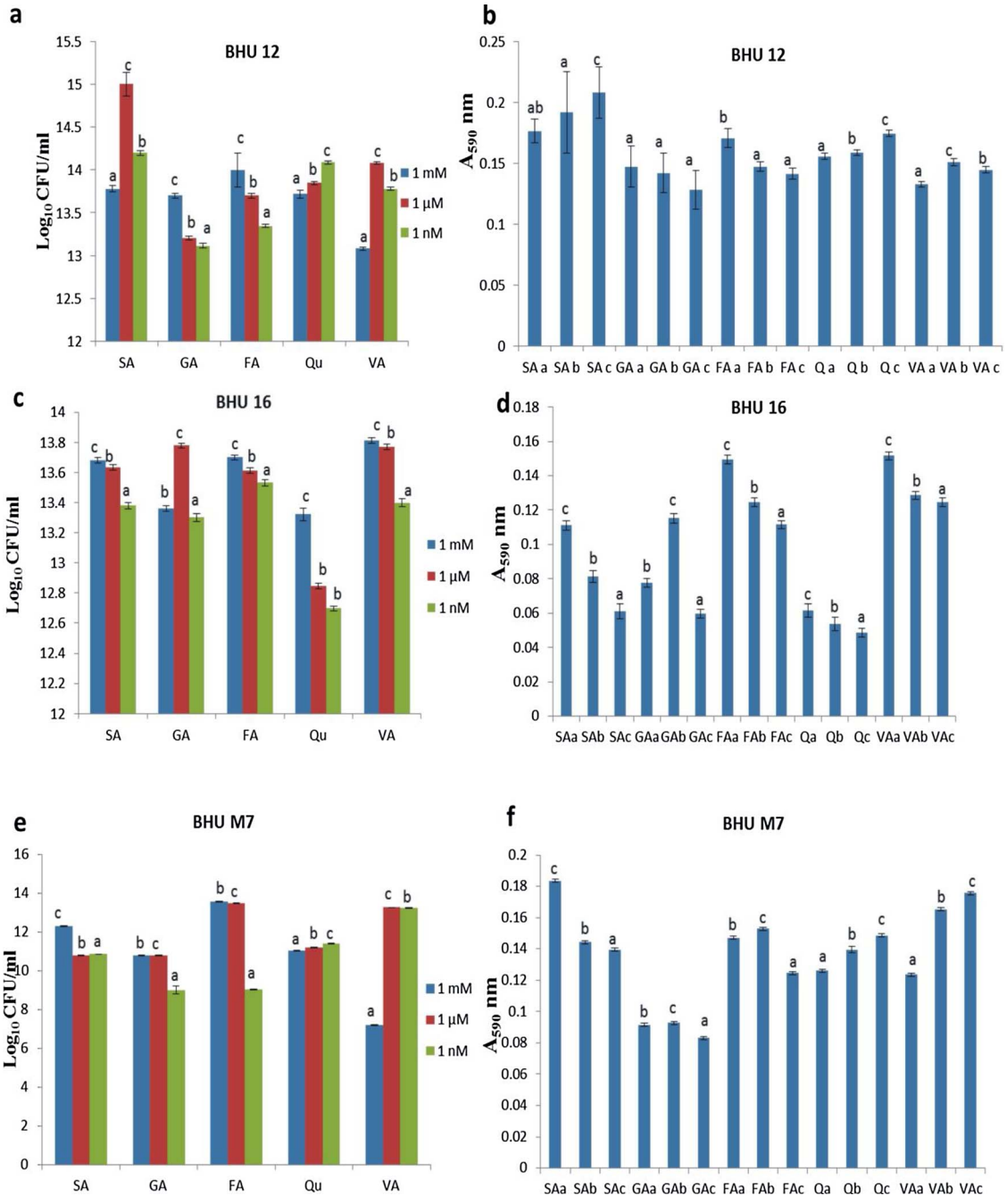

Fig. 3. Quantitative estimation of chemotaxis and biofilm formation of the endophytic isolates towards the different root exudate phenolic components.

were not only attracted by host root exudates but they also colonized the host rhizoplane region, from where they may possibly penetrate to the host interior (Yao and Allen, 2006). HPLC analysis of root exudate phenolic components revealed the presence of six phenolic acids, i.e. shikimic acid, gallic acid, vanilic acid, ferulic acid, quercetin and kaempferol. Shikimic, vanilic and ferulic acids at micromolar and millimolar concentrations specifically attracted the respective endophytic strains and enabled their colonization. Thus, a plausible conclusion may be inferred that seed priming with the above phenolics may enhance colonization by *A. faecalis* as suggested earlier by Singh et al. (2016b). Further, since phenols enhance induced systemic resistance during plant-phytopathogen interactions (Sarma et al., 2002; Singh et al., 2014), hence the current study indirectly authorizes that phenolic acid release in root exudates confirms augmented production of phenolics *in planta* due to *A. faecalis* colonization which further aids in enhanced growth and defense parameters of host plant as has been reported previously (Ray et al., 2015, 2016).

Upon colonization, the HPLC analysis of root exudates suggested a significant increase in gallic acid content in plants treated with BHU 12 while comparatively, a lower increment in gallic acid was observed in plants treated with BHU 16 and BHU M7. On the contrary, the concentration of gallic acid in the root exudates of treated plants declined upon pathogen challenge. A similar trend was also observed in case of shikimic acid concentration in root exudates of the plants treated with BHU 12, BHU 16 and BHU M7. This study plausibly suggests that an amelioration of shikimic acid pathway occurred due to treatment with the endophytic strains, thereby leading to an enhancement in growth and phenylpropanoid pathway as reported earlier (Ray et al., 2016). Decline in the level of shikimic acid following infection could be correlated to its transformation to other phenols having antimicrobial and

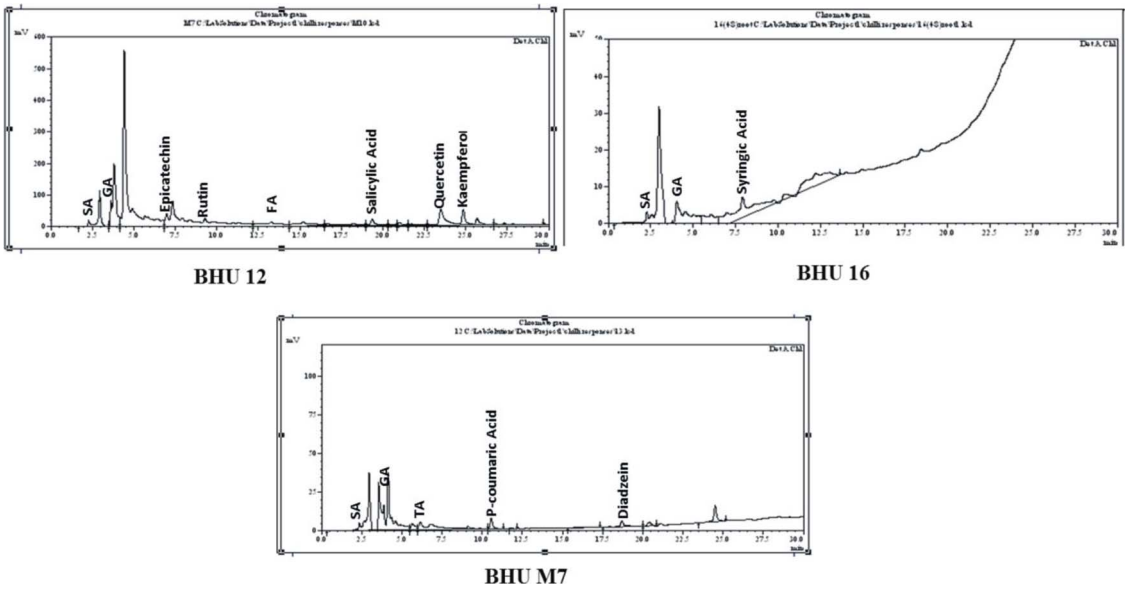

Fig. 4. HPLC chromatogram depicting exudation of phenolics by okra plants upon treatment with BHU 12, BHU 16 and BHU M7 (SA: shikimic acid; GA: gallic acid; FA: ferulic acid; TA: tannic acid).

antioxidant activities as also reported by Jain et al. (2015). This probably occurred to ensure a resilient form of protection by the host against the invading pathogen. According to Pusztahelyi et al. (2015), gallic acid in root exudates of *A.thaliana* significantly enhanced defense against *Fusarium* spp. Besides, gallic acid also supports lignification of plant tissues thereby restricting growth and ingress of pathogen. Since, the amount of phenolics produced *in planta* is at an elevated level as compared to the amount released in form of exudates (Verbon and Liberman, 2016), thus it can be predicted that an initial increment in phenylpropanoid pathway and lignin deposition was initiated upon treatment with the endophytic microbes thereby strengthening their internal defense mechanism.

Monolignol biosynthesis, the building block of lignin polymer, also requires *p*-coumaric acid and ferulic acid, apart from gallic acid. In the current study, plants primed with the three endophytic strains justify the above information. However, decline in the amount of the above mentioned phenolics in root exudates post infection may possibly relate to their increased metabolism *in planta* and thereby lower exudation in soil. Ferulic acid is also well known for its high antimicrobial nature (Sarma et al., 2002). Similarly, syringic, rutin and synaptic acid also act as effective antimicrobial agents. Our results demonstrating presence of syringic acid in root exudates of BHU 12 and BHU M7 primed plants are quite in concord with the above information as these phenolics released in the form of root exudates will act as a primary form of defense against *S. rolfsii*.

Nautiyal et al. (2002) reported that flavonoids, such as quercetin, myrecetin and kaempferol act as facilitators of plant–microbe interactions and root colonization by beneficial microbes. This report aptly

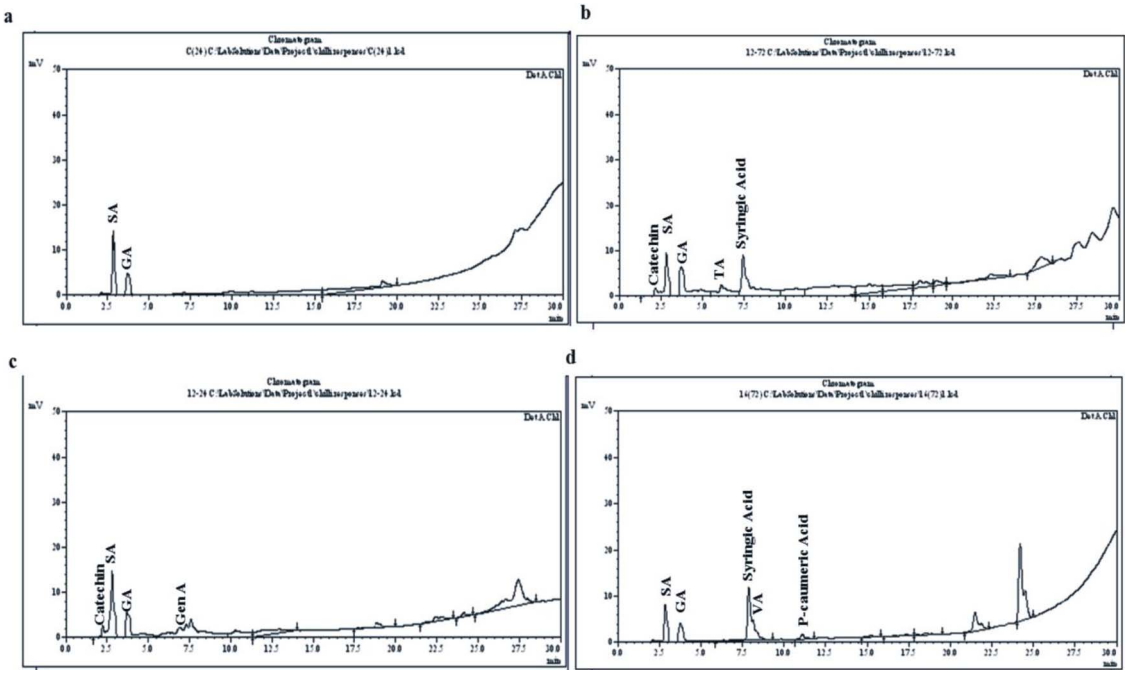

Fig. 5. HPLC chromatogram demonstrating exudation of phenolics by treatments primed with BHU 12 (b), BHU 16 (c) and BHU M7 (d) and challenged with *S. rolfsii* upon comparison with unprimed challenged control (a). GA = gallic acid; SA = shikimic acid; TA = tannic acid; VA = vanilic acid; Gen A = gentisic acid.

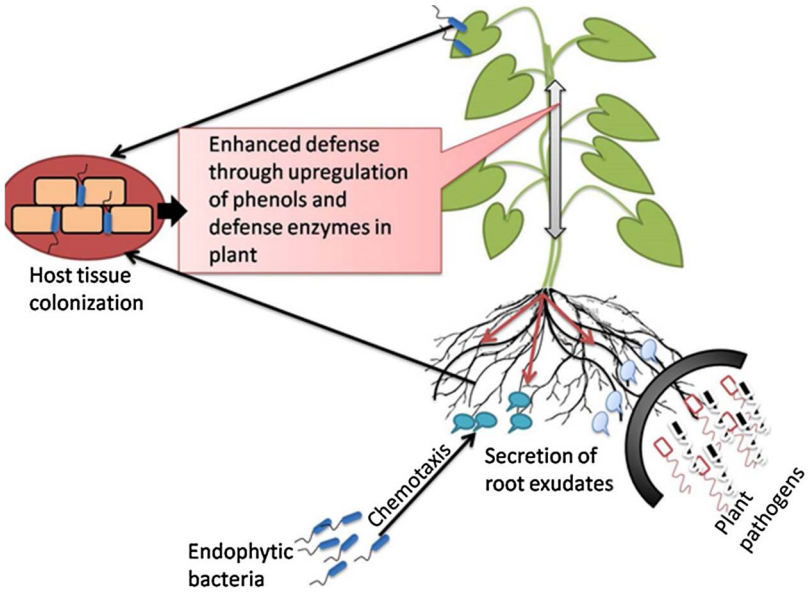

Fig. 6. Schematic representation of the careful manipulation of root exudates promoted by endophytic bacterial colonization for successful biotic stress management.

justifies our results as BHU 12 treated plants released quercetin and kaempferol in their root exudates plausibly to recruit more of these beneficial strains to the rhizoplane region. Further, according to Hassan and Mathesius (2012), induction of flavonoid biosynthesis occurs in response to effectors that contribute resistance against phytopathogens. In an earlier report, quercetin and kaempferol were found to restrict the growth of fungal phytopathogens, *viz. Pyricularia oryzae* and *R. solani* (Padmavati et al., 1997). According to Elad and Evensen (1995), epicatechin acts as an inhibitor of cell wall macerating enzymes and plant lipoxygenase. Thus, it can be concluded that epicatechin released upon treatment with BHU 12, possibly armours the host against any form of attack by fungal phytopathogens.

Quite significantly, daidzein though being a primarily leguminous plant borne flavonoid, was also observed in root exudates of plants treated with BHU M7. Daidzein is a central component of the isoflavonoid biosynthetic pathway and is the precursor of glyceollins, coumestrol and other bioactive flavonoids that have antifungal activity (Gutierrez-Gonzalez et al., 2010).

Presence of rutin in root exudates of BHU 12 treated plants plausibly suggests that the hosts have fortified themselves against the pathogenic rhizomicrobiota. Jain et al. (2015) also reported that rutin is quite significant during early defense response thereby justifying the above result. Salicylic acid plays a key role in both local and systemic resistance against plant pathogens and induces pathogenesis related (PR) proteins (Rivas-San Vicente and Plasencia, 2011). Increased amounts of salicylic acid in the present study can be correlated with the increase in the activity of PR proteins (Jain et al., 2012).

5. Conclusion

Phenolics in root exudates of *A. esculentus* not only attracted endophytic *A. faecalis* but also facilitated their colonization on the host rhizoplane. The microbes, post colonization, further ameliorated the inbuilt defense mechanism of the host thereby causing a significant alteration in phenolic composition of the host root exudate which evidently fortified the plant against any form of pathogen attack. The host root exudates exhibited a further amelioration upon receiving the *S. rolfsii* infection signals which suggests the sophisticated modification of host physiology directed by endophytic *A. faecalis* (Fig. 6). These findings can be directed towards understanding the mode of action of endophytic *Alcaligenes* sp. *in planta* which would further enable the preparation of a suitable bioformulation of the endophytic isolates for enhancement in sustainable agriculture.

Author contributions

The experiments were conceived and designed by HBS. Experiments were performed by SR and SM. Data interpretation was done by HBS, BKS, SS, and SR. Manuscript preparation was done by HBS, BKS, SS, SR, SM and KB.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

SR and KB are grateful to Banaras Hindu University for providing financial assistance in form of UGC-RET fellowship.

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