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Original article

Comparative analysis of endophytic bacterial diversity between two varieties of sunflower *Helianthus annuus* with their PGP evaluation

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ABSTRACT

Endophytic bacterial diversity shows an intricate network of interactions with host plants as they reside in various tissues and organs at certain stages or all stages of their life cycle stimulating the plant growth and fitness. Sunflower is a trendy oilfield crop and variation in its varieties is associated with the dynamics of endophytic diversity. The present study is undertaken to identify and compare the ecological niche of endophytic bacterial communities amongst different tissues of two hybrids varieties Hysun-33 and Hysun-39 of sunflower (*Helianthus annuus*) at three developmental stages which are vegetative stage I (after 15 days of seeds germination), vegetative stage II (after 30 days of germination) and reproductive stage (after 90 days of germination). A total of 74 endophytes from Hysun-33 and 115 endophytes from Hysun-39 have been isolated from different tissues and growth stages. Amongst plant parts, root tissues harbored higher bacterial inhabitants (44) followed by stem (33), leaf (30) and flower (7) of Hysun-39. Likewise, Hysun-33 endophytes colonized roots more abundantly followed by leaves, stem and flowers. All strains are found to be gram positive with the exception of only RA9 from Hysun-33 and RB9 from Hysun-39 that are gram negative. Among different growth stages, the maximum bacterial population (CFU of 320×10^3) was found amongst root microflora at vegetative stage II of plant in Hysun-39 variety as compared to root endophytes of Hysun-33 having (CFU of 10×10^3). The evaluation of their growth promoting features revealed that among 74 isolates of Hysun-33, 70% exhibited the ability of hydrogen cyanide production, 43% IAA production, 36% siderophore production and 4% nitrogen fixation and also phosphate solubilization. However among 115 isolates of Hysun-39, 64% appeared as hydrogen cyanide producers, 56% IAA producers, 33% siderophore producers, 2% nitrogen fixers and 4% as phosphate solubilizers. Therefore our study reveals understanding of wide-ranging diversity of endophytic bacteria and their beneficial relationship with internal tissues of host plant which may recommend their implementation to crops for better development of agricultural systems.

1. Introduction

Sunflower (*Helianthus annuus L.*) is a worldwide crop belonging to family Asteraceae and has been initiated from North America (Castro et al., 2018). It is also known for hybrid breeding as its hybrids have been found to upraise crop sustainability on worldwide scale (Skoric, 2012; Seiler et al., 2017). Plants are found to be associated with microbial populations as a host. This plant microbial association proves to be beneficial in improving the plant health (Mendes et al., 2013; Jones et al., 2019). Many studies have been conducted to explore the extent of microbial densities in plant environment. These microbiota make interactions with different plants and regulate their several physiological processes. In addition to this, various abiotic factors including soil characteristics may have influence on the plant microbiota associations (Santoyo et al., 2017). The most widely considered group of plant associated microorganisms are said to be endophytes that inhabits the internal plant tissues by means of leaves, root, stem, or seeds without triggering any injurious consequence on host plant (Yadav, 2018). Root hairs may serve as point of entry site for these endophytes. In this way intracellular plant-microbe interactions activate as they gain access to the root hairs (Preito et al., 2011;

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YU et al., 2019). These endophytes are capable of direct acquisition to the intracellular plant spaces for which they secrete various degrading enzymes for cell wall and establish endophytic root colonization association (White et al., 2014; Liu et al., 2017).

Among various microbes, endophytic bacteria have been found as a novel biopotential tool for providing extensive assistances to the agriculture. Essentially these microbes reside inside the plant tissues and enhance the growth of plants significantly by adopting various direct and indirect means of plant growth promotion. Such beneficial mechanisms include fixation of nitrogen biologically, solubilizing phosphorous, producing siderophores and HCN (Yadav, 2018). Large amount of research have been conducted on the study of biofertilizers which discloses that these microbes possess the aptitude of providing essential nutrients to the crops in adequate amount for the enrichment of crop yield (Suman, 2016; Yadav, 2018). Here we report the relative presence of endophytic bacteria in different parts of sunflower as well as developmental stages. Rare studies have been found to explore the diversity of endophytes and their role in plant growth promotion on two varieties (Hysun-33 and Hysun-39) of sunflower.

2. Materials and methods

The seeds of *Helianthus annuus* of two hybrid varieties naming Hysun-33 and Hysun-39 were procured from Imperial Chemical Industries (ICI) Lahore, Pakistan. These seeds were sown in a randomized row design pattern, maintaining the 25 cm distance between plants and 45 cm between rows on 27th of July, 2017 at The Women University, Multan. Physico-chemical properties of soil used for this experiment were; pH 8.9 (1:1 in H_2O), electrical conductivity 1.275 mS cm^{-1}, organic matter 0.40%, available potassium (K) 100 mg kg^{-1}, available phosphorous (P) 4.15 mg kg,$^{-1}$ available nitrogen (N) 0.60 mg Kg^{-1}, saturation 20% and soil textural class was sandy loam.

2.1. Isolation of endophytic bacteria

Roots, stem, leaves and flowers were washed with tap water to remove adhered soil particles. Surface disinfection of individual plant parts was accompanied with serial washing using 70% ethanol for 1 minute, 3% sodium hypochlorite for 1-2 minutes 70% ethanol for 30 seconds and finally with distilled water for 3-4 times. After sterilization, each plant part was macerated in a sterilized mortar and pestle. Each tissue sample was serially diluted in autoclaved water and spreaded on nutrient agar (peptone 10g/L, Beef 3g/L, agar 15g/L) plate for three days incubation at 37°C to isolates various endophytic bacteria. Colonies of bacteria were marked, isolated and further purified on selective media. These purified colonies were stored in nutrient agar slants at 4°C (Muzammal et al., 2012; Batool et al., 2016).

2.2. Morphological characterization with CFU count

Morphological features of bacterial isolates were recorded after 24 h of growth on nutrient agar medium by observing their color, shape, size, margins, elevation, texture and consistency along with the determination of colony forming unit per ml (CFU/ ml) (Riaz et al., 2015).

2.3. Biochemical identification of isolates

Different biochemical tests were performed such as gram staining, endospore staining, catalase utilization, starch hydrolysis, MR-VP and Simmons' citrate test for their identification. Gram staining was used to distinguish between gram positive and gram negative types on basis of their peptydoglycan content. Spore staining was used to identify *Bacillus* spp., *Corynbacterium* spp., *Lactobacillus* spp. and *Mycobacterium* spp. Catalase test was used to separate *Corynbacterium* spp. and *Lactobacillus* spp. Starch hydrolysis test was used to distinguish between *Corynbacterium kutsceri* and *Corynbacterium xerosis.* MR-VP test was performed to identify different *Bacillus* spp. (Riaz et al., 2009; Temitope et al., 2019).

2.4. PGP characterization of isolates

Bacterial isolates were screened for various plant growth promoting characteristics such as for auxin production, nitrogen fixation, phosphorus solubilization, siderophore production and hydrogen cyanide production (Batool et al., 2016). The determination of siderophore producing endophytes was done on Chrome Azurol S (CAS) agar by their spot inoculation on CAS agar plates which were incubated at 28 °C for 4 days. Development of orange-yellow halo around the growth was an indicator of siderophore producing bacterial isolates. For HCN production, the endophytes were streaked on nutrient agar plates supplemented with 0.4% (w/v) glycine which were covered with a filter paper soaked in picric acid solution (2% Na_2CO_3 in 0.5% picric acid) following 4 days of incubation for results (Gupta and Panday, 2019).

3. Results

3.1. Endophytic bacterial isolation and biochemical characterization of isolates

A total of 189 different bacteria were successfully isolated from leaves, stem, roots and flower tissues at different growth stages of plant (at vegetative stage I, vegetative stage II and at reproductive stage) considering its two varieties (Hysun-33 and Hysun-39) (Fig. 1).

The bacterial isolates from sunflower showed to have diverse characters comprising of colony size, shapes, elevation, texture, margins and colors (Supplementary Tables 1, 2, 3). Morphological and CFU count comparison of bacterial flora isolated from two varieties (Hysun-33 and Hysun-39) at the time of vegetative stage I revealed greatest diversity in root tissues of Hysun-39 (Fig. 2a).

Data collected at time of vegetative stage II showed that endophytes isolated from leaves and root tissues of Hysun-33 variety were higher than that isolated from stem tissues. However highest CFU count was recorded in root tissues and lowest were recorded in leaf tissues of Hysun-39 variety (Fig. 2b).

Data recorded at reproductive stage of plant showed greatest diversity of bacterial flora in leaf tissues of Hysun-33 and Hysun-39. At this stage the flower tissues also comprised of bacterial colonies but their number is lesser in flower tissues as compared to leaf tissues on the whole (Fig. 2c).

Regarding the biochemical tests, 95% and 96% positive results of Hysun-33 and Hysun-39 endophytes respectively had been recorded for gram staining and catalase tests at vegetative stage I. The percentage of spore producers was 60% for Hysun-33 endophytes and 65% for that of Hysun-39 endophytes. 80% of Hysun-33 isolates hydrolyzed starch while 75% of Hysun-39 endophytes gave positive results for the same test. MR-VP test revealed positive results for 75% of Hysun-33 and 69% of Hysun-39 endophytes. The Simmons citrate test was found to be positive for 65% of Hysun-33 and 72% of Hysun-39 endophytes (Fig. 3a).

At vegetative stage II, 87% and 78% gram positive rod shaped strains with only 12% and 21% gram positive cocci strains in Hysun –33 and Hysun-39 respectively were concluded. 62% of Hysun-33 and 69% of Hysun-39 were found to be spore formers. Regarding the catalase test, all endophytes of both varieties showed positive

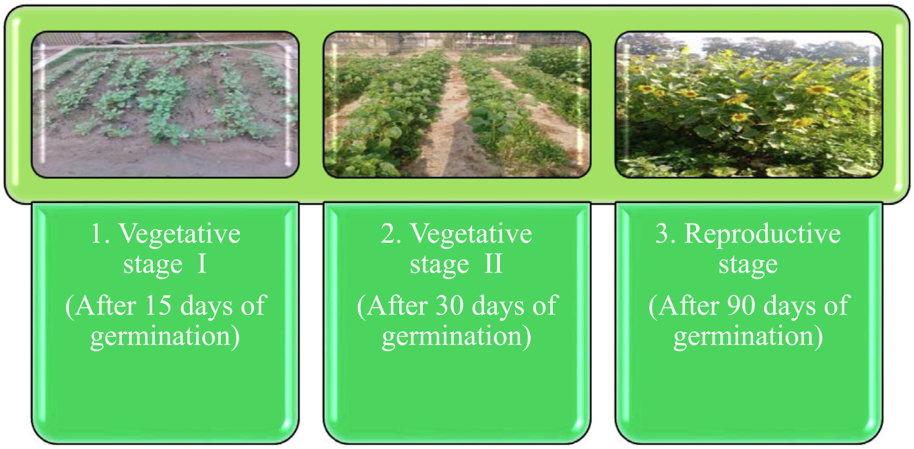


Fig. 1. Different growth stages of Sunflower *Helianthus annuus.*

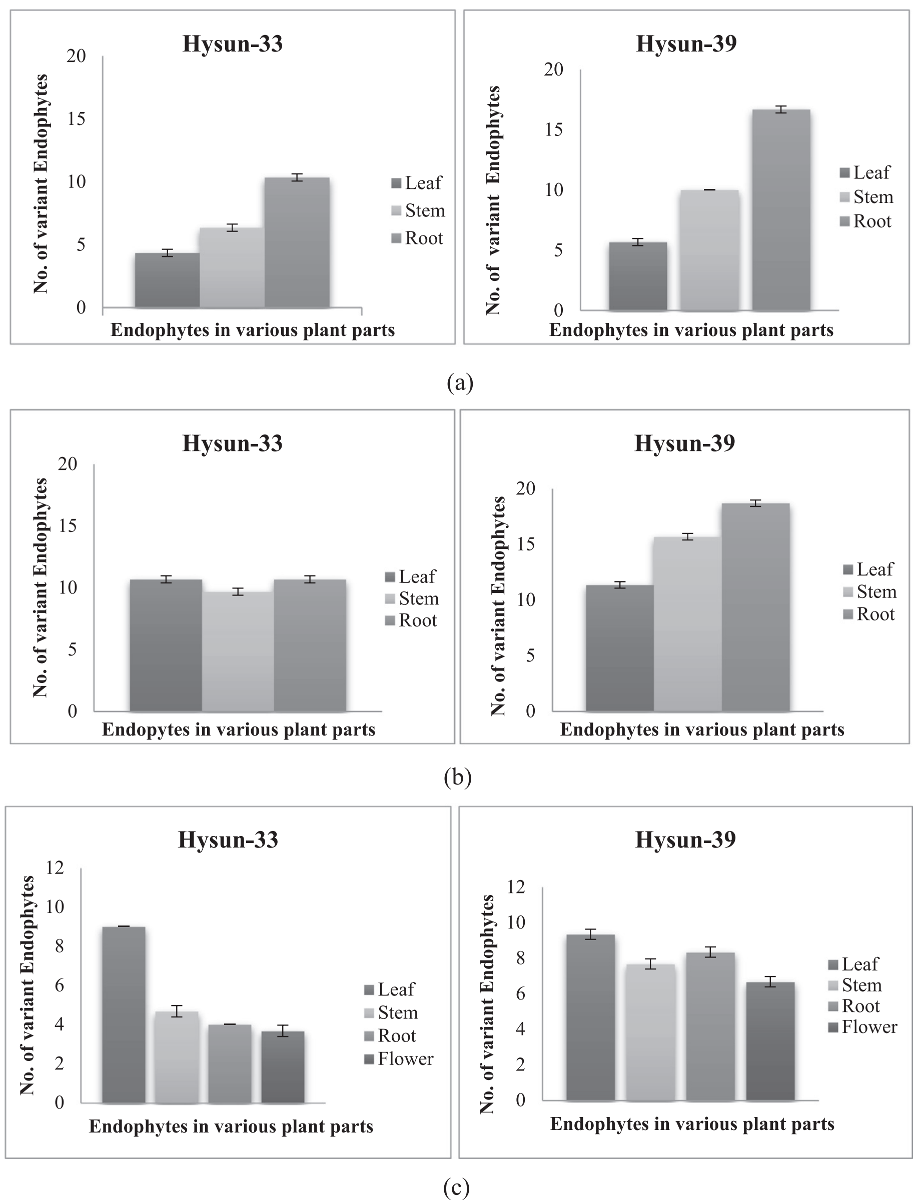
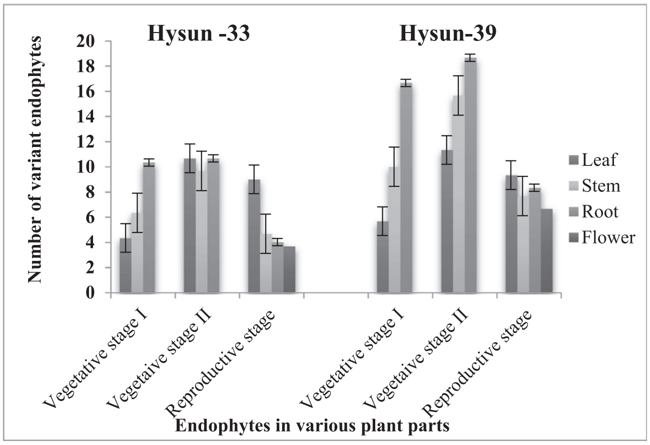


Fig. 2. Number of variant entophytic bacteria in various plant parts and at three growth stages (a). vegetative stage I (b). Vegetative stage II (c). reproductive stage of two varieties Hysun-33 and Hysun-39 (d). Comparison of bacterial endophytic diversity among two varieties.



(d)

Fig. 2 (*continued*)

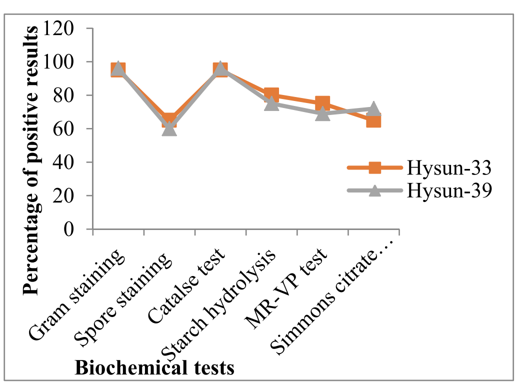
result with exception of only one named as 2RA11. 28% endophytes of two varieties produced starch. The strains tested for MR-VP test gave 65% positive results in Hysun-33 and 71% positive results in Hysun-39. However Simon's citrate test on these endophytes showed 68% and 69% positive results in respective varieties (Fig. 3b).

Results of biochemical tests recorded at reproductive stage revealed 100% positive results for gram staining and catalase test for endophytes of both varieties of plant (Hysun-33 and Hysun-39). While only 44% endophytes were found to be spore producers as compared to Hysun-33 endophytes (77%). The results of starch hydrolyzing endophytes were almost similar for both varieties (77% and 75% respectively). More endophytes (86%) of Hysun-33 gave positive results for MR-VP and Simmons citrate tests on comparison with Hysun-39 endophytes which were to be positive almost 75% for the same tests (Fig. 3c).

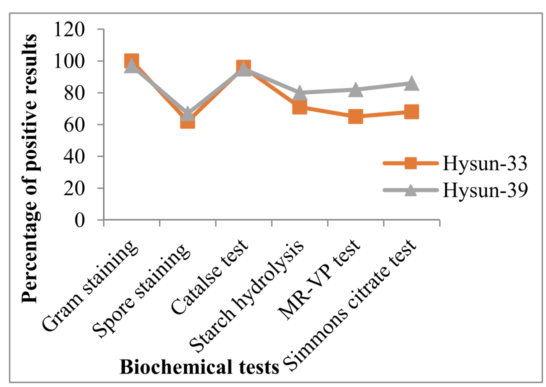
3.2. PGP characterization of endophytes

Majority of endophytes from leaves and stem tissues isolated from both varieties appeared as IAA producers. However only few root endophytes 1RA3, 1RB3 and 1RB12 gave positive result for this test. Among 53 isolates, only 3 isolates of leaves 1LA4 from Hysun-33 and 1LB4, 1LB6 from Hysun-39 exhibit the ability to fix nitrogen. Only two isolates 1LA4 and 1SA3 of Hysun-33 and 3 isolates 1LB4, 1LB6 and 1SB3 from Hysun-39 showed have efficiency of phosphate solubilization. Out of 53 isolates of both varieties, 9 isolates of Hysun-33 and 16 isolates of Hysun-39 were able to produce siderophores. Similarly among all isolates tested, 9 isolates from Hysun-33 and 13 isolates from Hysun-39 presented positive results for hydrogen cyanide production (Supplementary data).

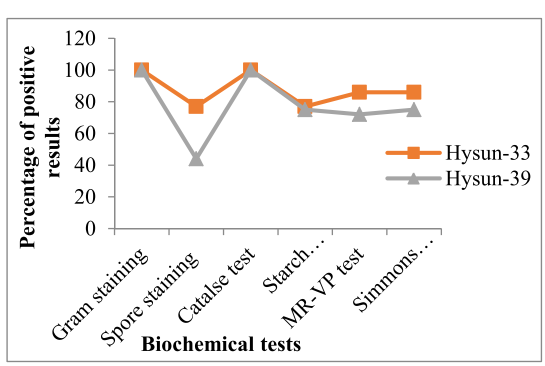
As total of 79 endophytes were isolated at the time of second vegetative stage and were tested for IAA production. Their result displayed that out total, only 8 isolates from Hysun-33 and 21 isolates from Hysun-39 showed positive results. Among isolates of Hysun-33, none of them showed the ability for nitrogen fixation and among isolates of Hysun-39, only one isolate 2SB15 showed its ability to fix nitrogen. Similarly only one isolate from each variety appeared to solubilize phosphate when tested which are 2RA7 and 2SB15 respectively. The siderophore production test on them also showed minor results. Only 9 isolates from Hysun-33 and 10



(a)



(b)



(c)

Fig. 3. Biochemical tests performed at three growth stages (a). vegetative stage I, (b).vegetative stage II, (c). reproductive stage of two varieties (Hysun-33 and Hysun-39) of sunflower.

isolates from Hysun-39 had the ability of siderophore production. 26 isolates of Hysun-33 were found as hydrogen cyanide producers and 6 isolates gave negative. However out of 46 isolates of Hysun-39, 33 showed positive result and only 13 were found to be negative.

The results of PGP tests of endophytes isolated at time of stage of plant that was the flowering/ reproductive stage were slightly different. Here majority of isolates of both varieties were IAA producers. However only few endophytes appeared as nonproducers of IAA. Regarding the results of nitrogen fixation and phosphate solubilition, majority of endophytes appeared to be negative for both tests. Only few of those that were 3LA1 and 3SA1 from Hysun-33 had ability to fix nitrogen by showing halo zones. Similarly only one isolate naming as 3RB2 showed halos around its colony for phosphate solubilization. Between the tests for siderophore and HCN production, HCN producers were found to be greater in number as compared to siderophore producers of both varieties.

Graphical representation (Fig. 4a–c.) of sunflower endophytes of both varieties (Hysun-33 and Hysun-39) showed that there are highest hydrogen cyanide producers and least nitrogen fixers and phosphate solubilizers. The average of auxin producers was also higher as compared to nitrogen fixers, phosphate solubilizers and siderophore producers but lesser than hydrogen cyanide producers. Another important aspect is that as compared to plant tissues, the leaf tissues produced maximum hydrogen cyanide producers at initial seedling stage. However there was an increasing trend of plant growth promoters in stem, root and flower tissues of both cultivars from vegetative to reproductive stage.

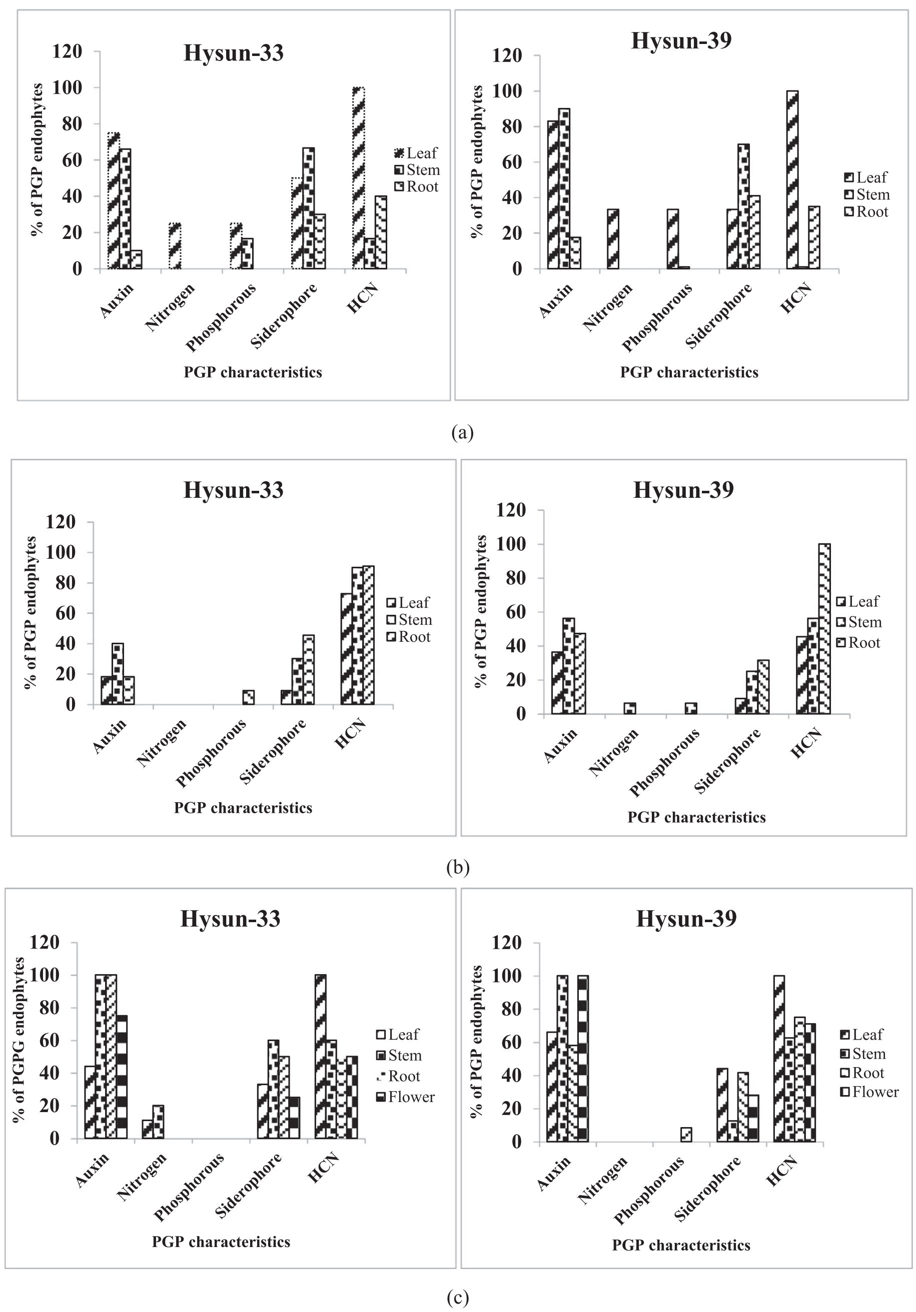


Fig. 4. Plant growth promoting (PGP) parameters at three growth stages (a). vegetative stage I, (b). vegetative stage II, (c). reproductive stage of two varieties (Hysun-33 and Hysun-39) of sunflower.

4. Discussion

Rare studies have been done on endophytic diversity of hybrid varieties (Hysun-33 and Hysun-39) of sunflower. The occurrence and diversity of bacterial endophytes from hybrid varieties of sunflower (*Helianthus annuus*), isolated under natural conditions have a great significance as these endophytes can impart their beneficial effect on plant's health. In the present study, a total of 189 bacterial endophytes have been reported from two varieties of plant considering three different growth stages. There is great dynamics in diversity of endophytic bacteria within different tissues and between both varieties of plant. The increasing trend of endophytic diversity has been found in root tissues as compared to leaf and stem tissues at vegetative stages I and II among both varieties. Abundance of bacterial population in root tissues could be due to the fact that they can exudate and digest diverse class of multifarious compounds. This idea is supported by Karnwal and Dohroo (2018). While at reproductive stage, the higher diversity of leaf isolates as compared to flower isolates had been found which might suggest shift of essential metabolites to leaf tissues required for plant growth. These results correspond with earlier findings of Shi et al. (2013); Elmagzob et al. (2019), showing greatest diversity of endophytic bacteria in leaf tissues. By comparing the entire endophytic bacterial flora of all the three growth stages, it had been found that root tissues occupy the highest diversity of bacterial endophytes. Similarly, Marag et al. (2018) reported maximum bacterial count in roots amongst different tissues in different growth stages of maize (*Zea mays* L.).

The morphological and biochemical account of the isolates exposed the supremacy of gram-positive bacteria over the gram-negative endophytes. However Tsegaye et al. (2018) found higher ratio of gram negative bacteria having 30% proportion and lower proportion of gram positive bacteria having only 10% proportion. Our study reported maximum endospore forming bacteria out of 189 isolates of both varieties. Being endospore formers these isolates may play significant role as biocontrol agents by antagonistically fighting with pathogens of host plant under natural environmental conditions. These results coincide with the results displayed by Melnick et al. (2011); Rabbee et al. (2019).

With reference to PGP results (98) IAA producing bacteria had been found from both crop types. These results suggest that IAA producing bacteria might increase the number of lateral roots which might exudate various minerals and nutrients required for plant growth. These findings coincide with the outcomes of Mike-Anosike et al. (2018) who reported six species of bacteria with the ability to produce 4.0–10 mg/L of IAA. Majority of the plants do not have the ability to consume nitrogen present in atmosphere unless it is converted into usable form of nitrogen by bacteria (Ahemad and Kibret, 2014). In our study, very few bacteria have been reported to fix nitrogen. Latt et al. (2018) postulated six bacteria as nitrogen fixers which supports our results. Phosphorus possess the seventeen number among essential plant nutrients but it is becoming a deficient factor for them. Agricultural crops have been reported to contain upto 0.5% of phosphorous only (Jayawardhane and Yapa, 2018). Our study reported only 8 phosphate solubilizing bacteria out of 190 isolates which may suggests that bacteria lacking phosphate solubilizing ability may adopt some other ways for plant growth promotion. However Zheng et al. (2018) isolated 76 bacteria having ability to solubilize phosphate from agricultural soil. Siderophores act as agents to chelate metals because of having low molecular weight. Plants and microorganisms release siderophores in iron deficient conditions (Chaudhary et al., 2017; Ferreira et al., 2019). Our report presented 65 siderophore producers out of 190 isolates which may suggest their role in reducing metals from plants and phytoremediation. These findings coincide with the report given by Grobelak and Hiller (2017).

Some bacteria promote the growth of plants indirectly by release of various chemicals essential to fight with plant diseases. Hydrogen cyanide is considered as an important metabolite that helps to combat various plant diseases by acting as a biocontrol agent. It actually stops electron transporting chain and inhibits energy source of the cell, causing death of pathogens (Ogale et al., 2018). Greater number of hydrogen cyanide producers (126) is presented by our study of isolates of two hybrid varieties that is supported by the findings of Tsegaye et al. (2019) who reported 38% hydrogen cyanide producing bacteria from the study of *Lolium Perrene*.

Therefore, isolation, identification and categorization of divergent endophytic microorganism isolated from altered niches with possible phytostimulent ability can support in improving the sustainable agricultural applications (Borah et al., 2018).

5. Conclusion

The study showed paradigm shift of endophytic bacterial diversity at differential growth stages of two hybrid varieties of sunflower *Helianthus annuus*. As the two varieties of plant exhibit abundance of beneficial microflora so these varieties may act as most promising varieties to increase the crop productivity. The colonization of miscellaneous endophytes, with the plant tissues reveal numerous activities for growth enhancement of host plant. Thus these endophytes can form a streamline association with multiple plant tissues and can be engaged as a source of biofertilizer for sustainable agriculture. Future research suggests to investigate the role of these endophytic communities in production of essential primary and secondary metabolites and in what mechanisms they impart their role in order to enhance the crop yield.

Declaration of Competing Interest

None.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2019.12.010.

References

Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J. King. Saud. Univ. Sci. 26, 1–20.

Batool, F., Rehman, Y. and Hasnain, 2016. Phylloplane associated plant bacteria of commercially superior wheat varieties exhibit superior plant growth promoting abilities. Front. Life. Sci. 9, 313–322.

Borah, M., Das, S., Boruah, H., Boro, R., Barooah, M., 2018. Diversity of culturable endophytic bacteria from wild and cultivated rice showed potential plant growth promoting activities. bioRxiv. doi.org/10.1101/310797.

Castro, C., Elite, R.M.V.B.C., 2018. Main aspects of sunflower production in Brazil. OCL, 25, doi.org/10.1051/ocl/2017056.

Chaudhary, D.Y., Gosavi, P., Durve-Gupta, A., 2017. Isolation and application of siderophore producing bacteria. I.J.A.R. 3, 246–250.

Elmagzob, A.H., Ibrahim, M.M., 2019. Seasonal diversity of endophytic bacteria associated with *Cinnamomum camphora* (L.) Presl. Abubakr. Diversity 11. https://doi.org/10.3390/d11070112.

Ferreira, C.M.H., Vilas-Boas, A., Sousa, C.A., Soares, H.M.V.M., Soares, E.V., 2019. Comparison of five bacterial strains producing siderophores with ability to chelate iron under alkaline conditions. AMB Expr. 9. https://doi.org/10.1186/s13568-019-0796-3.

Grobelak, A., Hiller, J., 2017. Bacterial siderophores promote plant growth: screening of catechol and hydroxamate siderophores. Int. J. Phytoremediation. 19, 825–833.

Gupta, S., Pandey, S., 2019. ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in french bean (*Phaseolus vulgaris*) plants. Front. Microbiol. 10, 1506. https://doi.org/10.3389/fmicb.2019.01506.

Jayawardhane, S., Yapa, N., 2018. Potential of microbial solubilization of rock phosphate for use in sustainable agriculture: does biochar application enhance microbial solubilization? J. Adv. Microbial. 8, 1–8.

Jones, P., Garcia, B.J., Furches, A., Tuskan, G.A., Jacobson, D., 2019. Plant host-associated mechanisms for microbial selection. Front. Plant Sci. 10. https://doi.org/10.3389/fpls.2019.00862.

Karnwal, A., Dohroo, A., 2018. Effect of maize root exudates on indole-3-acetic acid production by rice endophytic bacteria under influence of L-tryptophan. F1000Research.7, doi: 10.12688/f1000research.13644.1.

Latt, Z.K., Yu, S.S., Kyaw, E.P., Lynn, T.M., Nwe, M.T., et al., 2018. Isolation, evaluation and characterization of free living nitrogen fixing bacteria from agricultural soils in myanmar for biofertilizer formulation. Int. J. Plant Biol. Res. 6, 1092.

Liu, H., Carvalhais, L.C., Crawford, M., Singh, E., Dennis, P.G., Pieterse, C.M.J., Schenk, P.M., 2017. Inner plant values: diversity, colonization and benefits from endophytic bacteria. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02552.

Marag, P.S., Suman, A., Gond, S., 2018. Prospecting endophytic bacterial colonization and their potential plant growth promoting attributes in hybrid maize (*Zea mays* L.). Int. J. Curr. Microbiol. App. Sci. 7, 1292–1304.

Melnick, R.L., Suárez, C., Bailey, B.A., Backman, P.A., 2011. Isolation of endophytic endospore-forming bacteria from Theobroma cacao as potential biological control agents of cacao diseases. Biol. Cont. 57, 236–245.

Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbial. Rev. 37, 634–663.

Mike-Anosike, E.E., Braide, W., Adeleye, S.A., 2018. Studies on indole acetic acid (IAA) production by rhizobacteria and growth promoting potentials. Int. J. Adv. Res. Biol. Sci. 5, 133–140.

Muzammal, H., Sarwar, R., Sajid, I., Hasnain, S., 2012. Isolation, identification and screening of endophytic bacteria antagonistic to biofilm formers. Pak J Zool. 44, 249–257.

Ogale, S., Yadav, K.S., Navale, S., 2018. Screening of endophytic bacteria from the pharmacologically important medicinal plant *Gloriosa superba* for their multiple plant growth promoting properties. J. Pharm. Innov. 7, 208–214.

Prieto, P., Schilirò, E., Maldonado-González, M.M., Valderrama, R., Barroso-Albarracín, J.B., Mercado-Blanco, J., 2011. Root hairs play a key role in the endophytic colonization of olive roots by Pseudomonas spp. with biocontrol activity. Microb. Ecol. 62, 435–445.

Rabbee, M.F., Ali, Md.F., Choi, J., Hwang, B.S., Jeong, S.C., Baek, K.H., 2019. *Bacillus velezensis*: a valuable member of bioactive molecules within plant microbiomes. Molecules 24. https://doi.org/10.3390/molecules24061046.

Riaz, R., Sajjid, I., Sarwar, A., Rehman, F.U., Akhtar, M., Sadiq, S.A., Nassem, M., Ahmed, N., 2015. Molecular characterization and assessment of phylogenetic relationship among tomato rhizospheric bacterial population. Biologia (Pakistan) 61, 7–15.

Riaz, S., Ahmad, A., Hasnain, S., 2009. Antibacterial activity of soaps against daily encountered bacteria. Afr. J. Biotechnol. 8, 1431–1436.

Santoyo, G., Hernández-Pacheco, C., Hernández-Salmerón, J., Hernández-León, R., 2017. The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review, Span. J. Agric. Res. 15, doi.org/10.5424/sjar/2017151-9990.

Seiler, G.J., Qi, L.L., Marek, L.F., 2017. Utilization of sunflower crop wild relatives for cultivated sunflower improvement. Crop. Sci. 57, 1083–1101.

Shi, Y., Zhang, X., Lou, K., 2013. Isolation, characterization, and insecticidal activity of an endophyte of drunken horse grass *Achnatherum inebrians*. J. Insect Sci. 13. https://doi.org/10.1673/031.013.15101.

Skoric, Seiler, G.J., Liu, Z., Jan, C.C., Miller, J.F., Charlet, L.D., 2012. Sunflower genetics and breeding: international monograph/D. Novi Sad: Serbian Academy of Sciences and Arts. Branch, 520, 165–354.

Suman, A., Verma, P., Yadav, A.N., Srinivasamurthy, R., Singh, A., Prasanna, R., 2016. Development of hydrogel based bio-inoculant formulations and their impact on plant biometric parameters of wheat (*Triticum aestivum* L.). Int. J. Curr. Microbiol. App. Sci. 5, 890–901.

Temitope, O.O., Emmanue, K.T., 2019. Antibiotic resistant pattern of bacteria isolated from faecal pellets of *Supella longipalpa* (Cockroach) found in Akungba Akoko, Ondo state, Nigeria. J. Anal. Tech. Res. 1, 012–026.

Tsegaye, Z., Assefa, F., Tefera, G., Alemu, T., Gizaw, B., 2018. Characterization and identification of Tef (*Eragrostis tef*) seed endophytic bacterial species and evaluate their effect on plant growth promotion. J. Plant. Pathol. Microbiol. 9. https://doi.org/10.4172/2157-7471.1000438.

Tsegaye, Z., Gizaw, B., Tefera, G., Feleke, A., Chaniyalew, S., Alemu, T., Assefa, F., 2019. isolation and biochemical characterization of plant growth promoting (pgp) bacteria colonizing the rhizosphere of Tef crop during the seedling stage. BJSTR 14, 013–027.

White Jr, J.F., Torres, M.S., Somu, M.P., Johnson, H., Irizarry, I., Chen, Q., Bergen, M., 2014. Hydrogen peroxide staining to visualize intracellular bacterial infections of seedling root cells. Micros. Res. Tech. 77, 566–573.

Yadav, A.N., 2018. Biodiversity and biotechnological applications of host-specific endophytic fungi for sustainable agriculture and allied sectors. Acta. Sci. Microbiol. 1, 5. https://doi.org/10.31080/ASMI.2018.01.0044.

Yu, K., Pieterse, C.M.J., Bakker, P.A.H.M., Berendsen, R.L., 2019. Beneficial microbes going underground of root immunity. Plant Cell Environ. 42, 2860–2870.

Zheng, B.X., Ibrahim, M., Zhang, D.P., Bi, Q.F., Li, H.Z., Zhou, G.W., Ding, K., Peñuelas, J., Zhu, Y.G., Yang, X.R., 2018. Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. App. Ind. Microbiol. Biotech. Exp. 8. https://doi.org/10.1186/s13568-018-0575-6.