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# Spatio-temporal distribution of endophytes in tomato (*Solanum lycopersicum*) crop

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ABSTRACT

Endophytes considered another version of rhizosphere microbes, are associated with all types of plants. However, their population and diversity have greater consequences in terms of plant healthcare, the information on these is still little understood. Tomato growing areas covering 11 districts, representing four major agro-climatic regions of Assam were studied for spatio-temporal distribution of endophytes. A total of 177 bacterial endophytes and 57 fungal endophytes were isolated from tomato crop, out of which; 143, 51 and 40 isolates were obtained from roots, stems and leaves, respectively. The maximum of 112 endophytes were isolated during monsoon followed by 64 and 58 isolates, isolated during pre-monsoon and post-monsoon periods, respectively, indicating the rich diversity in bacterial and fungal endophytes of tomato crop. The maximum tissue colonization was observed in roots followed by leaves and stems in decreasing order, with *Bacillus* sp. amongst bacterial isolates and *Trichoderma* sp. amongst fungal isolates. These observations on spatio-temporal distribution of bacterial and fungal endophytes suggested their potential habitat and suitable time of studying endophytes for better healthcare of tomato crop.

**Keywords**: Assam, Distribution, Endophytes, Leaves, Monsoon, Roots, Stem, Tomato

The term “Endophyte” (Greek: endon=within and phyton=plant) was coined by Anton de Bary (1866) to describe fungi that colonizing internal tissues of plants. Since then, the concept of endophyte has undergone number of changes from time to time, based on origin of different microbial communities (predominantly contributed by fungal and bacterial species) and their relationship with host plant (Hallmann *et al.* 1997, Rosenblueth and Martinez-Romero 2006, Bora and Bora 2010, Bora *et al.* 2019, Bora *et al.* 2020). A more comprehensive definition of endophyte is described as; phylogenetically diverse microorganisms living within healthy tissue of plants that complete certain or entire life phases inside the host plant without causing any harmful effects to the host plants (Bora and Bora 2008, Bora *et al*. 2013). Endophytes usually live through symbiotic association with plants ranging from facultative saprobe, latent pathogen to mutualistic symbionts (Bora and Bora 2021, Bora *et al.* 2021). Plant-endophyte interactions influence plant productivity, plant diversity and plant pathogen interactions imparting a widespread interest in agro-ecological research (Van Bael *et al*. 2012, Borkotoki *et al*. 2016, Bora *et al.* 2020).

Many environmental factors affect plant-endophyte association, however the response of host plant towards endophyte infection also influence such association, mainly mediated by the host genotype, endophytic strain, resource availability etc. (Qawasmeh *et al*. 2012, Wani *et al*. 2015). Moreover, plants growing in different geographical regions are confronted with different environmental challenges (Arnold and Lutzoni 2007). Thus, the diversity of endophytes associated with the plant species varies with time and space (Ek-ramos *et al*. 2013), in addition to plant species (Bora *et al.* 2019). Even different plants growing in similar environmental conditions do not harbor same endophytes (Bora *et al.* 2016a). Many researchers have isolated different endophytes from various plant parts of tomato (Abbamondi *et al*. 2016, Abdallah *et al*. 2018, Lopez *et al*. 2018). More importantly, the endophyte microbial communities inhabit different plant structures, viz. leaves, petioles, reproductive structures, twigs, bark and roots (Rodriguez *et al.* 2009). Despite these breakthroughs, the crop specific distribution pattern of endophytes is highly lacking. In this background, the seasonal variation of endophyte population isolated from tomato and their tissue specificity in tomato plants were investigated. Such information would pave the way to identify promising endophytes capable of providing the tomato healthcare.

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## MATERIALS AND METHODS

*Exploration of tomato growing areas*: An extensive exploration of tomato growing areas, representing four agro-climatic zones of Assam, viz. i) central Brahmaputra valley zone (26.6528° N, 92.7926° E), ii) hill zone (25.3478° N, 93.0176° E), iii) upper Brahmaputra valley zone (27.4728° N, 94.9120° E) and iv) north bank plain Zone (NBPZ) (26.4014° N, 90.2667° E) and as many 9 districts (Nagaon, Karbi anglong, Jorhat, Majuli, Sivsagar, Golaghat, Sonitpur Lakhimpur, Dhemaji) was carried out. The tissue samples of tomato plants were collected as leaf, stem and root during pre-monsoon, monsoon and post-monsoon periods of 2019–20 to throw some valid lights on the seasonal variation of endophytic populations.

*Isolation and enumeration of tomato endophytes microflora*: Endophytic microflora associated different tissue of plant parts such as leaf, stem and root of healthy tomato plants was further subjected to analysis of endophytes. The processed plant parts of tomato (leaves, stem and root) were surface sterilized to eliminate epiphytes to ensure true endophytes count. The concentration of sterilizing agent and time of exposure were standardized to obtain maximum number of endophytes while ensuring no growth on sterility check. The plant samples (1 g) were exposed to 70% ethanol for 3 min and washed with sterilized water. The samples were again surface sterilized with 4% NaOCl for 1 min followed by washing thrice with sterile distilled water and 1 min exposure to 2% sodium thiosulphate containing 1% Tween 20. The samples were again washed three times with sterile distilled water and blot dried. The sterilized blot dried sample tissues (leaves, stem and roots) were placed in double autoclaved mortar and pestle containing 9 ml potassium phosphate buffer (PB, 0.1M) and homogenized thoroughly. The homogenate was then serially diluted in sterile water up to $10^{-7}$ for endophytic fungi and bacteria isolation using potato dextrose agar and nutrient agar medium media, respectively. Consequently, the distilled water used for the final wash was spread onto media, which serve as control (Schulz *et al.* 1993). All inoculated plates were incubated at 27 ± 2°C for 24 h to ensure the efficacy of surface sterilization. The data, thus, generated were analysed for coefficient of variation to establish the extent of diversity within plant parts and seasons.

## RESULTS AND DISCUSSION

*Endophytes diversity across seasons*: The distribution pattern of endophytic microorganisms showed a considerable variation in their population across different seasons. The highest population of endophytic microflora (112=33 fungi and 79 bacteria) was observed during monsoon followed by pre-monsoon (64=13 fungi and 51 bacteria) and post-monsoon (58=11 fungi and 47 bacteria). A highest number of 65 endophytes were recorded from Diphu, Karbi Anglong under hill zone. Highest bacterial endophytes (130) were recovered during monsoon and pre-monsoon (47) followed by post-monsoon (11). Similarly, recovery of fungal endophytes was highest in monsoon (33) followed by pre-monsoon (13) and post-monsoon (11), displaying quite an interesting pattern of distribution.

The higher diversity and population of endophytic microflora in pre-monsoon and monsoon was observed directly related to volume of precipitation coupled with humidity during these seasons. Earlier studies suggested that higher diversity and population of endophytic microflora were governed by higher precipitation levels (Bulgari *et al*. 2011, Zimmerman and Vitousek 2012, Suryanarayan 2013). The prevailing dry spell of weather mainly contributed towards lower population of endophytic microflora in post-monsoon characterized by much lower precipitation.

*Endophytes diversity across plant parts*: The diversity in endophytes, regardless of plant tissues was astonishingly very high, evident from coefficient of variation (Table 1), showing highest (143) numbers of endophytic fungi (34) and bacteria (109) confined to tomato root samples. Whereas,

Table 1 Spatial and temporal variation in plant parts derived endophytes from tomato growing areas of Assam

| Agro-climatic zones of Assam | Tomato growing location | Spatial distribution of endophytes | | | Temporal distribution of endophytes | | |
|---|---|---|---|---|---|---|---|
| | | Root | Leave | Stem | Pre-monsoon | Monsoon | Post-monsoon |
| Central Brahmaputra valley zone | Teliangaon,Panigaon and Nagaon | 15 | 4 | 3 | 7 | 9 | 6 |
| Hill zone | Karbi Anglong | 36 | 16 | 13 | 18 | 29 | 18 |
| Upper Brahmputra valley | Jorhat, Majuli, Sivasagar and Golaghat | 42 | 10 | 5 | 15 | 29 | 13 |
| North bank plain zone | Sonitpur, Lakhimpur, Gogamukh and Dhemaji | 50 | 21 | 19 | 24 | 45 | 21 |
| Total | - | 143 | 51 | 40 | 64 | 112 | 58 |
| (CV%) | - | 58.4 | 24.3 | 14.1 | 32.1 | 42.3 | 18.1 |

Total bacterial endophytes=177; Total fungal endophytes =57 with roots, stems and leaves tissues colonised at 61.11%, 17.09% and 21.79%, respectively, Frequency distribution of endophytes across 11 locations: Jorhat-34, Majuli-17, Sivsagar-0, Golaghat-6, Teliagaon, Nagaon-4, Panigaon, Nagaon-18, Sonitpur-3, Lakhimpur-30, Gogamukh, Dhemaji-26, Dhemaji-31, Karbi anglong-55.

tomato leaf and stem samples showed a comparatively lower degree of diversity out of total population of 51 (13 fungi and 38 bacteria) and 40 (10 fungi and 30 bacteria) endophytes, respectively. Both endophytic fungi and bacteria were recovered maximum from root samples (34 and 109, respectively) followed by leaves (13 and 38, respectively) and stem (10 and 30, respectively). District wise distribution of endophytes further showed no endophytic microorganisms recovered from the Sivsagar district due to samples collected during post-monsoon with moisture stress as one of the possible reasons. Such pattern of endophytes distribution needs to be looked into the observations made by Suryanarayan and Thennarasan (2004), who suggested that isolation of endophytic microflora may not be possible for a snapshot sampling.

Percent colonization of endophytes in tomato tissue was observed highest in roots (61.11%) followed by leaves (21.79%) and stem (17.09%). Highest number of endophytic fungi and bacteria were obtained from root suggesting maximum colonization in root samples (Table 1). Some of the earlier studies reported similar trend of colonization mentioning that acquisition of endophytes depended upon several physiochemical and biological factors, besides major habitat of endophytes as root tissues compared to other plant parts (Upreti and Thomas 2015, Bora *et al.* 2016b, Sahu *et al*. 2019). Infact, soil is the prime habitat of millions of micro-organisms confined to rhizosphere, playing a crucial role in ensuring robust plant health (Bora and Bora 2022). The composition and diversity of such microbes within a rhizosphere are associated with physiology of host plants in one of the three anticipated manners, viz. pathogenic, mutualist or commensalism (Saikia *et al.* 2020, Saikia *et al.* 2020). Endophytes make entry through entry sites, possibly as cracks near lateral roots, emergence, intercellular spaces as well as vascular bundles (Sharma *et al*. 2005).

Our observations on both, spatial as well as temporal distribution of 234 endophytes across 11 tomato growing districts of Assam showed of rich endophytes diversity existed within root tissues during monsoon period in order to harness full potential of endophytes for health management of tomato crop.

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## REFERENCES

Abbamondi G R, Tommonaro G , Weyens N, Thijs S, Sillen W, Gkorezis P, Iodice C, de Melo Rangel W, Nicolaus B and Vangronsveld J. 2016. Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. *Chemical and Biological Technologies in Agriculture* **3**(1): 1–10.

Abdallah R A B, Jabnoun-Khiareddine H, Stedel C, Nefzi A, Papadopoulou K K and Daami-Remadi M. 2018. Tomato-associated endophytic bacteria with Fusarium wilt suppression and tomato growth promotion abilities. *Journal of Agricultural Science and Food Research* **9**: 4–12.

Arnold A E and Lutzoni F. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* **88**(3): 541–49.

Bora L C and Bora Popy. 2010. *Pseudomonas fluorescens* Pf D-1 based biopesticide 'Biofor Pf' for management of wilt disease of tomato and 'Bhootchilli. *Non-Chemical Insect Pest Management*, pp. 149–155. Ignacimuthu S and David B V (Eds). Elite Publishing House Pvt. Ltd, New Delhi.

Bora L C and Popy Bora. 2008. Vemicompost-based bioformulation for management of bacterial wilt of tomato in polyhouse. *Journal of Mycology Plant Pathology* **38**(3): 527–30.

Bora Popy and Bora L C. 2022. Revisiting non-chemical modes of diseases and pests management in tea (*Camellia sinensis*): a review. *Indian Journal of Agricultural Science* **92**(1): 03–09.

Bora Popy, Bora L C and Begum M. 2013. Eco-friendly management of soil borne diseases in brinjal through application of antagonistic microbial population. *Journal of Biological Control* **27**(1): 29–34.

Bora Popy, Bora L C and Bhuyan R P. 2021. Evaluation of botanicals and microbial bioformulations against grey blight disease of tea under organic production system. *Indian Journal of Agricultural Science* **91**(01): 54–57.

Bora Popy, Bora L C and Deka P C. 2016a. Efficacy of substrate based bioformulation of microbial antagonists in the management of bacterial diseases of some solanaceous vegetables in Assam. *Journal of Biological Control* **30**(1): 49–54.

Bora Popy, Bora L C, Deka P C, Bikran B, Sarmah A K, Dutta H S and Dabara B. 2016b. Efficacy of *Pseudomonas fluorescens* and *Trichoderma viride* based bioformulation for management of bacterial wilt disease of ginger. *International Journal of Plant Science* **11**: 180–86.

Bora Popy, Bora L C and Gogoi M. 2020. Potential of Trichoderma spp for pest management and plant growth promotion in NE India. *Advances in Trichoderma Research*, pp. 205–20. P. Sharma and Anil Sharma (Eds), Springer Publication, New York, USA.

Bora Popy and Bora L C. 2021. Microbial antagonists and botanicals mediated disease management in tea, *Camellia sinensis* (L.) Kuntze: an overview. *Crop Protection* **148**: 105711.

Bora Popy, Saikia K and Ahmed S S. 2020. Pathogenic fungi associated with storage rot of *Colocasia esculanta* and evaluation of bioagents against the pathogen. *Pest Management of Horticultural Ecosystem* **26**(1): 134–39.

Bora Popy, Saikia K, Hazarika H and Ragesh G. 2019. Exploring potential of bacterial endophytes in disease management of horticultural crops. *Current Horticulture* **7**: 32–37.

Borkotoki B, Sarma K K, Deka P C, Bora Popy and Sarmah A K. 2016. Combined effect of integrated nutrient management and site specific nutrient management using leaf colour chart in rainfed rice. *Annals of Plant Soil Research* **18**(4): 391–95.

Bulgari D, Casati P, Crepaldi P, Daffonchio D, Quaglino F , Brusetti L and Bianco P A. 2011. Restructuring of endophytic bacterial communities in grapevine yellowa-diseased and recovered *Vitis vinifera* L. plants. Applied Environment. *Microbiology* **77**(5): 44–54

Ek-Ramos M J, Zhou W, Valencia C U, Antwi J B, Kalns L L, Morgan G D and Sword G A. 2013. Spatial and temporal variation in fungal endophyte communities isolated from cultivated cotton (*Gossypium hirsutum*). *PloS One* **8**(6):e66049.

Hallmann J, Quadt-Hallmann A, Mahaffee W and Kloepper J. 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* **43**(10): 895–914.

López S M Y, Pastorino G N, Franco M E E, Medina R, Lucentini C G, Saparrat M C N and Balatti P A. 2018. Microbial endophytes that live within the seeds of two tomato hybrids cultivated in Argentina. *Agronomy* **8**(8): 136.

Qawasmeh A, Obied H K, Raman A and Wheatley W. 2012. Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: interaction between Lolium perenne and different strains of *Neotyphodium lolii*. *Journal of Agricultural and Food Chemistry* **60**(13): 3381–88.

Rodriguez R, White Jr J, Arnold A E and Redman A R A 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**(2): 314–30.

Rosenblueth M and Martinez-Romero E. 2006. Bacterial endophytes and their interactions with hosts. *Molecular plant-microbe interactions* **19**(8): 827–37.

Sahu P K, Singh Udai B, Chakdar H and Bagul S Y. 2019. Bacterial endophytes in agriculture: concepts to application- A training manual. Publisher: ICAR- National Bureau of Agriculturally Important Microorganisms, Kushmaur, Maunath Bhanjan, Uttar Pradesh (India), pp. 1–123

Saikia K, Bora L C, Bora Popy and Hazarika H. 2020. Management of bacterial blight of rice (*Oryza sativa*) through combined application of endophytes and rhizosphere antagonist. *Indian Journal of Agricultural Science* **90**(12): 2323–27.

Saikia S, Bora Popy and Bora L C. 2021. Bioagent-mediated canker management of Citrus canker. *Indian Journal of Agricultural Science* **91**(12): 198–201.

Sharma D, Kaur T, Kaur A and Manhas Rm K. 2005. Antagonistic and plant growth promoting activities of endophytic and soil actinomycetes. *Archives of Phytopathology and Plant Protection* **46**(14): 1756–68

Sharma P, Bora L C, Acharjee S, Bora Popy and Jagdale B R. 2020. Zinc enriched Pseudomonas fluorescens triggered defense response in rice against bacterial leaf blight. *Indian Journal of Agricultural Science* **90**(3): 593–96.

Suryanarayanan T S. 2013. Endophyte research: going beyond isolation and metabolite documentation. *Fungal Ecology* **6**(6): 561–68

Suryanarayanan T S and Thennarasan S. 2004. Temporal variation in endophyte assemblages of Plumeria rubra leaves. *Fungal Diversity* **15**: 197–204

Upreti R and Thomas P. 2015. Root associated bacterial endophytes from *Ralstonia solanacearum* resistant and susceptible tomato cultivars and their pathogen antagonistic effects. *Frontiers in Microbiology* **6**: 255–59

Van Bael S A, Estrada C, Rehner, S A, Santos J F and Wcislo W T. 2012. Leaf endophyte load influences fungal garde development in leaf-cutting ants. *BMC Ecology* **12**(1): 23.

Wani Z A, Ashraf N, Mohiuddin T and Riyaz-Ul-Hassan S. 2015. Plant-endophyte symbiosis, an ecological perspective. *Applied Microbiology and Biotechnology* **99**(7): 2955–65.

Zimmerman N B and Vitousek P M. 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. *Proceedings of the National Academy of Science* **109**(32): 13022–27.