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DIVERSITY AND DISTRIBUTION OF ENDOPHYTIC MICROORGANISMS IN TOMATO PLANTS IN KERALA

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ABSTRACT
This study investigated the distribution of endophytic microorganisms in stem and roots of tomato plants in Kerala. Dilutions of 10^{-4} and 10^{-3} which showed maximum countable colonies were selected for the isolation of endophytic bacteria from root and stem respectively, 10^{-2} and 10^{-1} dilution for endophytic fungi and actinomycetes respectively from both root and stem. Samples collected from various locations totally yielded bacterial, fungal and actinomycetes population of 1351 x 10^4 cfu g^{-1}, 272 x 10^2 cfu g^{-1}, 18 x 10^1 cfu g^{-1} and 832 x 10^3 cfu g^{-1}, 229 x 10^2 cfu g^{-1}, 3 x 10^1 cfu g^{-1} in root and stem respectively. From the enumerated microbial population, the predominant colonies of 154 endophytic microorganisms consisting of 79 bacteria, 68 fungi, and seven actinomycetes were obtained.

KEYWORDS: Diversity, distribution, endophytes, tomato, Kerala.

INTRODUCTION

The term 'endophyte' is derived from two Greek words, 'endon' meaning 'within' and 'phyton' meaning 'plant'. Endophytes are microorganisms that inhabit for at least one period of their lifecycle inside plant tissues without causing any apparent harm to the hosts (Petrini, 1991) and they benefit the host by promoting plant growth and prevent pathogenic organisms from colonization. Inside the host, endophytes have greater access to nutrients and a comfortable habitual niche (Ting *et al.*, 2008). The beneficial effects that the endophytes can confer on plants have made their role highly significant in biological control of diseases in various crops.The diversity and distribution of endophytic bacteria was first observed by Gardner *et al*. (1982), who identified bacteria present in the xylem fluid from the roots of the rough lemon rootstock and among the 13 genera, the most frequently occurred ones were *Pseudomonas* (40%) and *Enterobacter* (18%). Recent studies have revealed the ubiquity of the endophytic fungi residing within the plants. Xia *et al.* (2011) reported the distribution of various species of endophytic and epiphytic *Trichoderma* in banana roots with the largest population comprised of *T. asperellum*, *T. virens*, and *Hypocrea lixii*. Nimnoi *et al*. (2010) used PCR denaturing gradient gene electrophoresis to determine diversity and community of endophytic actinomycetes distributed within the roots of eagle wood and confirmed the presence of endophytic actinomycetes of genera *Nocardia, Pseudonocardia, Streptomyces* and *Actinomadura* within the roots. Therefore, an attempt was carried out to determine the diversity and distribution of endophytic microorganisms present in tomato plants in Kerala.

MATERIALS AND METHODS

Standardization of dilution factor for the isolation of endophytic microorganisms

Healthy tomato plants adjacent to wilted ones were uprooted, brought to the laboratory, and washed under running tap water to remove the soil particles adhering to the root. The root portion 5 cm below the soil line and the stem portion 10 cm above the soil line were taken for the isolation. The skin of the stem was peeled off and the root skin scraped off to remove the external contaminants and these were cut into bits of 1 cm length.

Isolation of endophytic bacteria

Endophytic bacteria were isolated from root and stem samples as suggested by McInroy and Kloepper (1995).The stem and root bits of one gram were weighed separately. Stem samples were disinfested with 20 per cent hydrogen peroxide and root samples with 1.05 per cent sodium hypochlorite for 10 min and rinsed four times with sterile 0.02 M tris phosphate buffer (pH 7). An aliquot of 1 ml of the final buffer wash was transferred to sterile Petri plate to which nutrient agar (NA) was added and it served as sterility check. Each sample was triturated in 9 ml of final buffer wash using a sterile pestle and mortar and dilutions were prepared up to 10^{-6} from this triturate. One ml from each dilution was pipetted into sterile Petri plates and 15 ml each of molten and cooled NA and King's B media were poured separately and the plates were incubated at room temperature (28 ± 2^0C) for 48 h.

Isolation of endophytic fungi

The isolation of endophytic fungi from stem and root samples were carried out according to Haiyan *et al.* (2005). One gram each of stem and root samples were surface sterilized separately by sequentially treating with 0.5 per

cent sodium hypochlorite and 70 per cent ethanol for 2 min, and rinsed with two changes of sterile water followed by two changes of sterile 0.02 M tris phosphate buffer. Sterility checks were kept on Martin's Rose Bengal Agar (MRBA) mediated plates. The samples were then triturated in 9 ml of final buffer wash and serial dilutions were prepared up to 10^{-3}. From each dilution, 1 ml was poured into sterile Petri plates and 15 ml each of molten and cooled MRBA and Trichoderma Selective Medium (TSM) were poured separately and the plates were incubated at room temperature for 72 h.

Isolation of endophytic actinomycetes

Endophytic actinomycetes were isolated from root and stem samples adopting the protocol of Tan *et al.* (2006). The stem and root bits of one gram were weighed separately and the root samples were surface sterilized with 70 per cent ethanol for 30 sec, followed by 1.05 per cent sodium hypochlorite for 5 min and the stem bits with 20 per cent hydrogen peroxide for 10 min. The samples were then soaked in 10 per cent sodium bicarbonate solution for 10 min and, rinsed four times in 0.02 M sterile phosphate buffer. Sterility checks were maintained on Kenknight's Agar medium (KAM). Each sample was ground in 9 ml of final buffer wash and dilutions up to 10^{-3} were prepared. One ml from each dilution was pipetted into Petri plates to which 15 ml of molten and cooled KAM was poured and the plates were incubated at 28 ± 2^0C for seven days.

Microbial population in each dilution was recorded and the number of colonies in each sample was calculated using the following equation.

$$\text{Number of cfu/g sample} = \frac{\text{Number of colonies x dilution factor}}{\text{Volume plated (ml)}}$$

Collection of samples

A purposive survey was conducted for the collection of the samples. Healthy tomato plants were collected from 16 different locations representing north, central and south Kerala namely Padanakkad (Kasaragod District), Panniyur (Kannur), Malappuram and Tavanur (Malappuram), Ozhalapathy and Eruthempathy (Palakkad), Vellanikkara, Mannuthy and Cherumkuzhy (Thrissur), Kalamassery (Ernakulam), Kumarakom (Kottayam), Alappuzha and Kayamkulam (Alappuzha), and Kallambalam, Vellayani and Amburi (Thiruvananthapuram).

Isolation of endophytes from the samples of different locations

The endophytic microorganisms *viz.* bacteria, fungi and actinomycetes were isolated from the samples collected from different locations using the standardized dilution factors for each organism and as per the protocols mentioned earlier.

The predominant microbial colonies were selected, purified and the pure cultures were maintained on potato dextrose agar (PDA).

RESULTS

Standardization of dilution factors for the isolation of endophytic organisms

Preliminary isolation of endophytic bacteria, fungi and actinomycetes from root and stem samples of tomato plant were carried out using Vellanikkara samples, on their selective media, adopting standard protocols and the dilution factors for the isolation of endophytes were standardized. Dilutions of 10^{-4} and 10^{-3} which showed maximum countable colonies were selected for the isolation of endophytic bacteria from root and stem respectively, 10^{-2} and 10^{-1} dilution for endophytic fungi and actinomycetes respectively from both root and stem (Table 1).

TABLE 1. Standardisation of dilution factor for the isolation of endophytic organisms

Dilutions	Endophytic population (cfu g^{-1} sample)					
	Bacteria		Fungi		Actinomycetes	
	Root	Stem	Root	Stem	Root	Stem
10^{-1}	Too numerous to count	Too numerous to count	29	22	3	1
10^{-2}	Too numerous to count	112	18	13	1	0
10^{-3}	180	44	2	0	0	0
10^{-4}	66	12	-	-	-	-
10^{-5}	19	3	-	-	-	-
10^{-6}	3	0	-	-	-	-

Isolation and enumeration of endophytic microbial population from collected samples

Endophytic microorganisms were isolated from both root and stem of healthy tomato plant samples collected from 16 locations. Quantitative estimation of the endophytic microorganisms was carried out from these 32 samples, using the dilution factors standardized for each type of microorganism and data are presented in Table 2. The isolated microbial population varied with the plant samples and the population was higher in root as compared to stem samples. Microbial population varied significantly with the samples collected from different locations. Samples collected from various locations totally yielded bacterial, fungal and actinomycetes population of 1351 x 10^4 cfu g^{-1}, 272 x 10^2 cfu g^{-1}, 18 x 10^1 cfu g^{-1} and 832 x 10^3 cfu g^{-1}, 229 x 10^2 cfu g^{-1}, 3 x 10^1 cfu g^{-1} in root and stem respectively. Only Vellanikkara and Amburi samples showed the presence of all three types of microorganisms in both root and stem.

TABLE 2. Endophytic microbial population of tomato collected from different locations

Sl. No	Location	Bacteria (cfu g^{-1})		Fungi (x 10^2 cfu g^{-1})		Actinomycetes (x 10^1 cfu g^{-1})	
		Root (x 10^4)	Stem (x 10^3)	Root	Stem	Root	Stem
1	Padannakkad (P)	68^{ef}	54^{bcd}	12^{d}	12^{de}	0	0
2	Panniyur (Py)	92^{d}	61^{abc}	26^{b}	13^{cde}	5	0
3	Malappuram (M)	52^{g}	44^{de}	22^{b}	12^{de}	0	0
4	Tavanur (T)	51^{g}	32^{fg}	14^{cd}	20^{ab}	0	0
5	Ozhalapathy (O)	82^{de}	68^{a}	14^{cd}	12^{de}	5	0
6	Eruthempathy(E)	73^{ef}	29^{g}	17^{c}	13^{cde}	0	0
7	Vellanikkara (V)	66^{ef}	44^{de}	18^{bc}	13^{cde}	3	1
8	Mannuthy (My)	63^{fg}	52^{cd}	13^{cd}	12^{de}	0	0
9	Cherumkuzhy (C)	71^{ef}	68^{a}	14^{cd}	15^{cde}	0	1
10	Ernakulam (Ek)	79^{de}	46^{de}	16^{cd}	23^{a}	0	0
11	Kumarakom (Ku)	138^{b}	64^{ab}	38^{a}	23^{a}	0	0
12	Alappuzha (A)	194^{a}	52^{cd}	14^{cd}	12^{de}	0	0
13	Kayamkulam (K)	118^{c}	64^{ab}	16^{cd}	11^{e}	0	0
14	Kallambalam (Ka)	80^{de}	46^{de}	14^{cd}	16^{bcd}	0	0
15	Vellayani (Vy)	52^{g}	68^{a}	12^{d}	11^{e}	0	0
16	Amburi (Am)	72^{ef}	40^{ef}	12^{d}	11^{e}	5	1
	Total	1351	832	272	229	18	3

Treatment means with same alphabet in superscript, do not differ significantly

Among the endophytic microorganisms isolated, bacterial population was higher than fungi and actinomycetes in both root and stem. Bacterial population varied from 51 to 194 x 10^4 cfu g^{-1} in root and 29 to 68 x 10^3 cfu g^{-1} in stem samples among the different locations. Maximum population was recorded in the root sample collected from Alappuzha (194 x10^4 cfu g^{-1}) followed by Kumarakom (138 x 10^4 cfu g^{-1}) and in case of stem, samples collected from Cherumkuzhy, Ozhalapathy and Vellayani harboured maximum of 68 x 10^3 cfu g^{-1}.Quantitative estimation of endophytic fungi revealed that, the population was comparatively less in root samples from all locations except in Kumarakom, Panniyur, and Malappuram which varied from 12 to 38 x 10^2 cfu g^{-1} root with maximum (38 x 10^2 cfu g^{-1}) in Kumarakom sample and in case of stem samples, it varied from 11 - 23 x 10^2 cfu g^{-1} with maximum in Ernakulam and Kumarakom (23 x 10^2 cfu g^{-1}). As compared to bacteria and fungi, actinomycete population was very less and only the root samples from Panniyur, Ozhalapathy, Amburi and Vellanikkara and the stem from Cherumkuzhy, Amburi and Vellanikkara showed actinomycete population. Among the different locations, the maximum population was present in root samples collected from Panniyur, Ozhalapathy and Amburi (5 x 10^1 cfu g^{-1}).From the enumerated microbial population, the predominant colonies of 154 microorganisms consisting of 79 bacteria, 68 fungi, and seven actinomycetes were selected. Among these, 44 bacteria, 42 fungi and four actinomycetes were from root and 35, 26 and three respectively from stem samples. Among the fungal endophytes, *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp were the predominant ones. There were five fluorescent pseudomonads among the isolated endophytic bacteria.

DISCUSSION

In order to study the diversity and distribution of endophytes in tomato, plant samples were collected from 16 different locations representing north, central and south Kerala and the endophytes were isolated from both stem and root of tomato plants. As a preliminary experiment, the dilution factors for the isolation of different endophytic organisms were standardised with Vellanikkara sample and 10^{-3} and 10^{-4} dilutions were found to be ideal for the isolation of endophytic bacteria from stem and root respectively. Likewise, 10^{-2} was standardized as dilution factor for isolating fungi whereas 10^{-1} for actinomycetes from both stem and root. However, Nawangsih *et al.* (2011) could isolate endophytic bacteria with 10^{-5} dilution from the stem and Nandhini *et al.* (2012) isolated with dilution of 10^{-10} from the roots and stem of tomato. Kurian (2011) also used higher dilutions of 10^{-7}, 10^{-4} and 10^{-5} for the isolation of endophytic bacteria, fungi and actinomycetes respectively from cacao root and shoot. The dilution factor may vary with plant species. Similarly, Mathew (2007), Uppala (2007), and Balan (2009) used10^{-2} dilution for the isolation of endophytic bacteria and fungi from stem and root of black pepper, amaranth and anthurium respectively. Majority of the reports on isolation of endophytic actinomycetes showed 10^{-1} dilution being the ideal for their isolation (Cao *et al.*, 2005; Tan *et al.*, 2006; Sreeja, 2011).Quantitative estimation of endophytic microorganisms in tomato from different locations revealed that, the bacterial endophytes formed the major population compared to fungi and actinomycetes. This result is in accordance with earlier reports by Kloepper *et al.* (1980), Fisher *et al.* (1992), Uppala (2007), Balan (2009) and Kurian (2011). Endophytes originate from rhizosphere or phyllosphere and they enter the endosphere mainly

through natural openings (Ryan *et al*., 2008). Since major share of microbial population of rhizosphere or phyllosphere is contributed by bacteria, it is reasonable to expect more bacteria in the endosphere also. It is similar to that observed by the earlier workers. It was also noticed that, root portion yielded high population of endophytes compared to stem which is in agreement with the findings of Tripathi *et al*. (2006), Mathew (2006), Rajendran *et al.* (2006), Balan (2009) and Kurian (2011). Yang *et al.* (2011) recorded 72 endophytic bacteria from tomato including 45 from stem and 27 from leaves. Patel *et al.* (2012) isolated 18 endophytic bacteria from root and stem of tomato plants collected from different regions of Gujarat. The present study yielded 79 bacteria, 68 fungi and seven actinomycetes from root and stem of tomato plants collected from different parts of Kerala.Variation in the population of endophytes was noticed among the samples collected from different locations. High fungal population was observed in both root and stem samples collected from Kumarakom, whereas bacteria was more in root samples from Alappuzha and the population of actinomycetes was maximum in Panniyur, Ozhalapathy and Amburi root samples. Since the endophytic population is influenced by external factors (Wilson and Carroll, 1994), samples collected from different agroclimatic locations resulted in the variation of endophytic population. This is in line with the observations of Uppala (2007), Balan (2009), Sreeja (2011) and Kurian (2011) who also noticed variation in the endophytic population in the samples collected from different locations of Kerala.

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