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POPULATION VARIATION OF ENDOPHYTIC ACTINOMYCETE IN SOYBEAN (*GLYCINE MAX* (L) MERRIL)

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ABSTRACT

Present investigation was carried out to study variation in endophytic actinomycete population colonizing soybean cultivated in vertisol. Endophytic population was assessed at different growth stages of soybean (C.V. JS-335) viz., vegetative and reproductive stages. A 186 (25.86 %) isolates were obtained from vegetative growth stages (V1-V5) and 533 (74.13 %) from reproductive growth stages. As plant grows the endophytic population increases progressively however, at the onset of reproductive stages viz., R1-R8 the endophytic population starts to decrease and attains a stable value at R8 stage. The maximum endophytic population was found at V4-V5 stages (1.83 ± 0.05 and 1.89 ± 0.06) and R1-R3 stages (1.80 ± 0.03, 1.78 ± 0.04 and 1.74 ± 0.03) at which the plant attains maturity. Our investigation provides a valuable insight in understanding of endophytic microorganisms in their unique ecological habitat.

INTRODUCTION

Plants present an excellent ecosystem for microorganisms. Plant-microbial interactions have been large area of research interest since long and may range from beneficial to harmful. Microbial endophytes are typically defined as plant associated microbes that colonize living internal tissues of plants without causing any visible symptoms or immediate over-negative effects and can be isolated from surface disinfected plant tissue (Wilson, 1995, Hung and Annapurna, 2004). Virtually all plants are hosts to endophytic microorganisms and endophytes may usually be fungi, bacteria and actinomycetes (Pimentel *et al.,* 2006). These microorganisms include both commensal species, which have no direct effect on the host plant, and mutualistic symbionts (Procopio *et al.,* 2009). Intimate associations between endophytes and host plants can be formed without harming the plant and they have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens and/or various environmental stresses (Hasegawa *et al.,* 2006). Endophytes have been shown to confer fitness benefits to host plants including tolerance to herbivory, heat, salt, disease and drought, and increased below and aboveground biomass. Thus, endophytic colonization improves the ecological adaptability of the host.

Endophytic actinomycetes have been isolated from within live tissues of various plant species (El-Tarabily *et al.,* 2009 and Rosenblueth and Martinez-Romero, 2006). Endophytic actinomycetes have been shown to protect plants against different soil-borne plant pathogens (Hasegawa *et al.,* 2006). The study of the structure of endophytic microbial population is important for understanding their ecological role in nature. Analysis of the structure of microbial populations has practical importance; the results can be used to assess the fate of released strains and their impact on resident microbial communities (Procopio *et al.,* 2009). Present investigation was carried out to study variation in endophytic actinomycete population colonizing soybean cultivated in vertisol.

MATERIALS AND METHODS

Sample collection

Healthy plants of soybean (C.V.JS-335) were screened from the different locations of Washim district (M. S., India) during the cultivation period June-December 2010. The soybean growth stages were identified as specified by McWilliams *et al.,* 2004. Sample represent of each growth stage viz., vegetative (V1-V5) and reproductive (R1-R8) were collected. The plants were uprooted, sealed into plastic bags and labeled. All samples were processed immediately after collection.

Isolation and identification of endophytic actinomycete

The collected plants were washed under tap water to remove soil and separated into root, stem and leaf. All root, stem and leaf samples were washed twice in distilled water then surface sterilized by immersion for 1 minute in 70% (v/v) ethanol, in 0.1% $HgCl_2$ upto 3 minutes for roots and nodules, whereas, upto 5 minutes for leaves and stems respectively. The tissue was then washed ten times using sterile distilled water. Sterility checks after surface sterilization were carried out by monitoring separately the section impressions and rinse wash water for the presence or absence of microbial growth upto 6 days on

selective media. The absence of growth was taken into consideration as positive test for surface sterilization (Hung and Annapurna, 2004).

The surface disinfected samples were macerated (Hallmann *et al.,* 1997). After filtering the slurry through sterile cotton cloth, the filtrate was serially diluted and enriched in glycerol yeast extract broth supplemented with soybean plant extract (@ 5 %) for 24h at 30ºC and aliquots (0.1mL) of enriched broth were spread with a sterile glass rod over the surface of glycerol yeast extract agar supplemented with aureomycin 10μg/mL. Plates were dried in a laminar air flow cabinet for 15 min before incubation at 28ºC in the dark for 15 days. Population densities were expressed as $\log_{10}$ colony forming units (CFU) g^{-1} fresh weight (El-Tarabily *et al.,* 2009). The isolates were identified to genus level according to *Bergey's Manual of Determinative Bacteriology* (Holt *et al.,* 1994).

Statstical analysis

The results were analyzed by standard statistical methods (Mungikar, 1997) and ANOVA single factor ($p < 0.05$) using software QI-Macros, 2012.

RESULTS AND DISCUSSION

Endophytic actinomycetes were isolated from different growth stages of soybean cultivated in vertisol. A 186 (25.86 %) isolates were obtained from vegetative growth stages (V1-V5) and 533 (74.13 %) from reproductive growth stages (Fig. 1). A 287 (40 %) isolates were isolated from roots, 245 (34 %) from leafs and 187 (26 %) from stem. Thus, roots as a primary site of entry shows maximum population followed by leaf and stem. About 87 % of the isolates were presumed to be in genus *Streptomyces* and rest of 13 % were found to be belonged to *Nocardia, Actinomadura Microbispora* and *Actinoplanes*. It has been found that the numbers of isolates obtained from vegetative stages were comparatively larger than that of reproductive stages.

As plant grows the endophytic population increased progressively. In vegetative stages viz., V1-V5 the endophytic population was found to be 1.59, 1.70, 1.76, 1.83 and 1.89 $\log_{10}$ CFU g^{-1} fresh weight respectively. However, at the onset of reproductive stages viz., R1-R8 the endophytic population decreased and attained a stable value at R8 stage, the endophytic population was found to be 1.80, 1.78, 1.74, 1.70, 1.66, 1.61, 1.56 and 1.48 $\log_{10}$ CFU g^{-1} fresh weight respectively. This represents that the endophytic population declined both in quantity and variety as plant aged. The maximum endophytic population was found at V4-V5 (1.83 and 1.89 $\log_{10}$ CFU g^{-1} fresh weight) and R1- R3 (1.80, 1.78 and 1.74 $\log_{10}$ CFU g^{-1} fresh weight) at which the plant attains maturity, this suggest that the matured plant has optimum population.

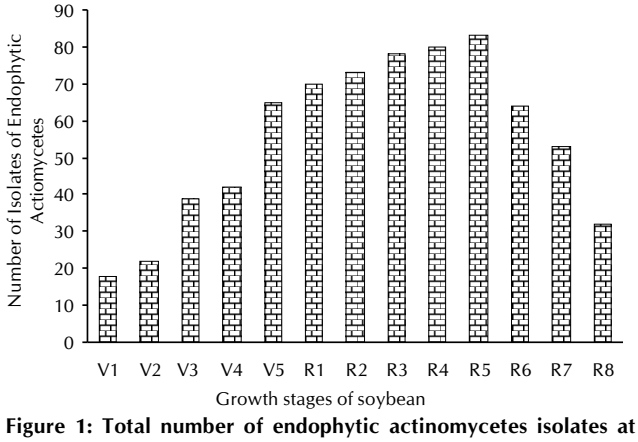

Figure 1: Total number of endophytic actinomycetes isolates at different growth stage of soybean

* Values presented are mean values of three independent experiments

The endophytic population is highly variable and occasionally transient (Hardoim *et al.,* 2008). The observed differences in endophytic population in our study is supported by other observation where (Kuklinsky-Sobral *et al.,* 2004) endophytic bacterial population densitites are found to be influenced by soybean growth phase and tissue sampled and Pimentel *et al.,* 2006 pointed that as plant ages the number of endophytic fungal isolates decreases. El-Tarabily *et al.,* 2009 also observed initial increase in endophytic actinomycetal population in cucumber roots and the population densities were found to be 4.56 $\log_{10}$ CFU g^{-1} fresh root weight As plant matures all the nutritional requirements for actinomycetes are optimum and a stable endophytic population is obtained thus, there appear to be coincidence of plant maturity and endophytic population. The endophytic population is influenced by several factors viz., the developmental stage during which the plant is sampled, environmental conditions and the geographical location of the plant (Kuklinsky-Sobral *et al.,* 2004). Moreover, the genotype as well as cultivar of plant also affects significantly as the endophytic population is naturally selected by host. Conn and Franco, 2004 also reported that soil type, to a large extent, determines the endophytic population. The decrease in endophytic population from vegetative to reproductive stages could have been due to a lack of essential nutrients. Pimental *et al.,* 2006 and McInroy and Kloeper, 1995 also observed that some of the essential nutrients needed by such bacteria were unavailable during the maturation and senescence of plants.

An understanding of the factors affecting the population of

Table 1: Total number of endophytic actinomycetes ($\log_{10}$ CFU g^{-1} fresh weight)

Growth stage	Number of endophytic actinomycetes				
		Plant source			Mean ± SE_M
		Root	Stem	Leaf	
Vegetative	V1	1.71	1.44	1.64	1.59 ± 0.09
	V2	1.79	1.56	1.76	1.70 ± 0.08
	V3	1.85	1.65	1.80	1.76 ± 0.06
	V4	1.90	1.73	1.86	1.83 ± 0.05
	V5	1.97	1.79	1.92	1.89 ± 0.06
Reproductive	R1	1.86	1.75	1.81	1.80 ± 0.03
	R2	1.85	1.71	1.78	1.78 ± 0.04
	R3	1.80	1.68	1.76	1.74 ± 0.03
	R4	1.76	1.63	1.71	1.70 ± 0.04
	R5	1.72	1.61	1.67	1.66 ± 0.03
	R6	1.66	1.57	1.62	1.61 ± 0.02
	R7	1.64	1.51	1.53	1.56 ± 0.04
	R8	1.61	1.50	1.34	1.48 ± 0.08

* Values presented are mean values of three independent experiments, Mean ± SE_M ($p < 0.05$).

endophytic actinomycete is necessary to achieve a consistent application of these microorganisms in relation to plant health and biocontrol. Research on endophytic actinomycete offers an innovative and enlivening opportunity for the discovery of novel strains with biotechnological utility.

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