

Comparative study of epiphytic and endophytic bacteria associated with seeds of *Zea mays* L.

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ABSTRACT

Maize (*Zea mays*.L) is a third most important cereal grain cultivated throughout the world, contributing 782 mt for world cereal production. The seeds of maize are the source for next generation and is associated with plethora of microorganisms both externally and internally. the present study aims at isolation and comparison of epiphytes and endophytes of seed associated bacterial communities of different maize genotypes in the premise of Tamil Nadu Agricultural University, Coimbatore. The beneficial aspects of epiphytic and endophytic bacteria have potential to establish the plant microbiome through vertical transmission. The transmission of microorganisms from parents to progeny having positive influence on plant health and fitness which confirms the presence of symbiotic partners. The colonization frequency of both epiphytes and endophytes differs greatly whereas the diversity and abundance of bacterial population was higher in epiphytes than in endophytes. This study revealed the existence of significant differences in bacterial population between the epiphytic and endophytic microorganisms.

Key words: Maize seed, Epiphytes and endophytes, Colony morphology

Introduction

Seed harbor diverse group of bacterial communities present on their surface and also reside inside the seed tissues evolved during germination and seedling development stages which governs the structure of plant microbiome. The microorganisms found on the surface of the seed are epiphytes and those microorganisms colonizes the inner tissues are endophytes. These microorganisms are non-pathogenic and play a significant role in promoting the plant health and functioning. Maize (*Zea mays*.L) is a coarse cereal crop domesticated throughout the world with a production of 783 mt (Parihar *et al.*, 2011). Nowadays, the production was affected by various biotic and abiotic factors. To combat the effect in eco-friendly way, the microorganisms associ-

ated with seeds found to be the better strategy in alternative to the chemical pesticides. The present study was to identify the similarity and differences between the epiphytes and endophytes isolated from three different genotypes (Hybrid, male and female) of maize seeds and evaluating its colonization and morphological characteristics among the parental and hybrid lines.

Materials and Methods

Maize seeds of three different genotypes Maize UMI-1230 (male), UMI-1200 (female), Maize COH6 (hybrid) were collected from Department of millets, Tamil Nadu Agricultural University, Coimbatore. The seed epiphytes and endophytes were isolated by serial dilution method. For epiphytes, the collected

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seeds were washed with detergent Phosphate buffer saline (PBS) and the epiphytes were suspended in the PBS wash. 1mL of the wash was used to make dilutions upto (10^{-4}) and was poured on different media for obtaining as much as possible isolates. For endophytes, the seeds were first surface sterilized aseptically with 0.1 % mercuric chloride for 30 sec followed by 70% ethanol wash for 2-3 min and then the seeds were rinsed with sterile distilled water for 2-3 times (Abiola and Oyetayo, 2016). The surface sterilized seeds were macerated with phosphate buffer saline in sterile pestle and mortar and 1mL of the macerated wash is used to make dilutions up to (10^{-4}) using pour plate method.

The media used for isolation of epiphytes and endophytes were Nutrient Agar (NA), Luria Bertani Agar (LB), Tryptic Soy Agar (TSA), Reasoners 2 A agar (R2A), Soil Extract agar (SEA), Starch casein agar (SCA). The plates were incubated for 2 days at $28\pm 2^{\circ}\text{C}$ (War Nongkhlaw *et al.*, 2014). After incubation, the plates were observed for colony development and the morphologically different colonies were picked and purified by repeated streaking on respected media. The epiphytic and endophytic plates of male, female and hybrid seeds were compared morphologically and colony characters were recorded.

Data analysis

The colony forming units of bacterial species was calculated according to (Motyl *et al.*, 2005) $\text{CFU/ml} = (\text{No. of colonies} \times \text{Total dilution factor}) / \text{Volume of culture plate in ml}$, where CFU is Colony forming

Units.

Results

The epiphytic and endophytic microflora of three different genotypes of Maize UMI-1230 (male), UMI-1200 (female), Maize COH6 (hybrid) was shown in Table 1. In epiphytic bacterial flora of hybrid seeds, the highest bacterial population of 2.31×10^6 cfu's/g of seed was recorded on NA followed by 1.30×10^6 on LB, 8.3×10^4 on TSA and the population was gradually declined in R2A, SEA and SCA as in Figure 1A. The viable count of epiphytic male seeds was greater in R2A with 16.2×10^4 cfu's/g of seed followed by 11.5×10^4 in TSA and count was decreased in LB and NA was shown in Fig 1B, whereas for epiphytic female seeds, the highest population count of 15.5×10^3 was observed in R2A followed by 14.4×10^3 in LB (Fig 1C). In case of endophytes, the highest bacterial count of 5.5×10^5 cfu's/g of seed was observed in NA plates of hybrid seeds followed by 2.3×10^5 in LB and population count was gradually reduced in TSA, SEA and R2A as in Fig 1A. On observing the endophytic male seed population, the highest viable count was recorded in NA with 4×10^4 cfu's/g of seed followed by 3.3×10^4 in LB whereas in endophytic female seeds, the greater population count of 14×10^3 cfu's/g of seed was observed in TSA, 12×10^3 in NA.

Discussion

The present study provides the basic understanding

Table 1. Epiphytic and Endophytic microflora of three different maize seed genotypes

Name of the sample	Medium used	Epiphytes Population (cfu/g of seed)	Endophytes Population (cfu/g of seed)
Maize COH6(Hybrid seed)	NA	2.31×10^6	5.5×10^5
	LB	1.30×10^6	2.3×10^5
	TSA	8.3×10^4	7×10^4
	SEA	4.36×10^4	10×10^3
	R2A	1.74×10^4	2×10^3
	SCA	2.18×10^4	2×10^3
Maize UMI-1230(Male seed)	NA	6.7×10^4	4×10^4
	LB	8.3×10^4	3.3×10^4
	TSA	11.5×10^4	3.1×10^4
	R2A	16.2×10^4	9×10^3
	NA	14.4×10^3	12×10^3
Maize UMI-1200(Female seed)	LB	11.8×10^3	5×10^3
	TSA	9.6×10^3	14×10^3
	R2A	15.5×10^3	11×10^3

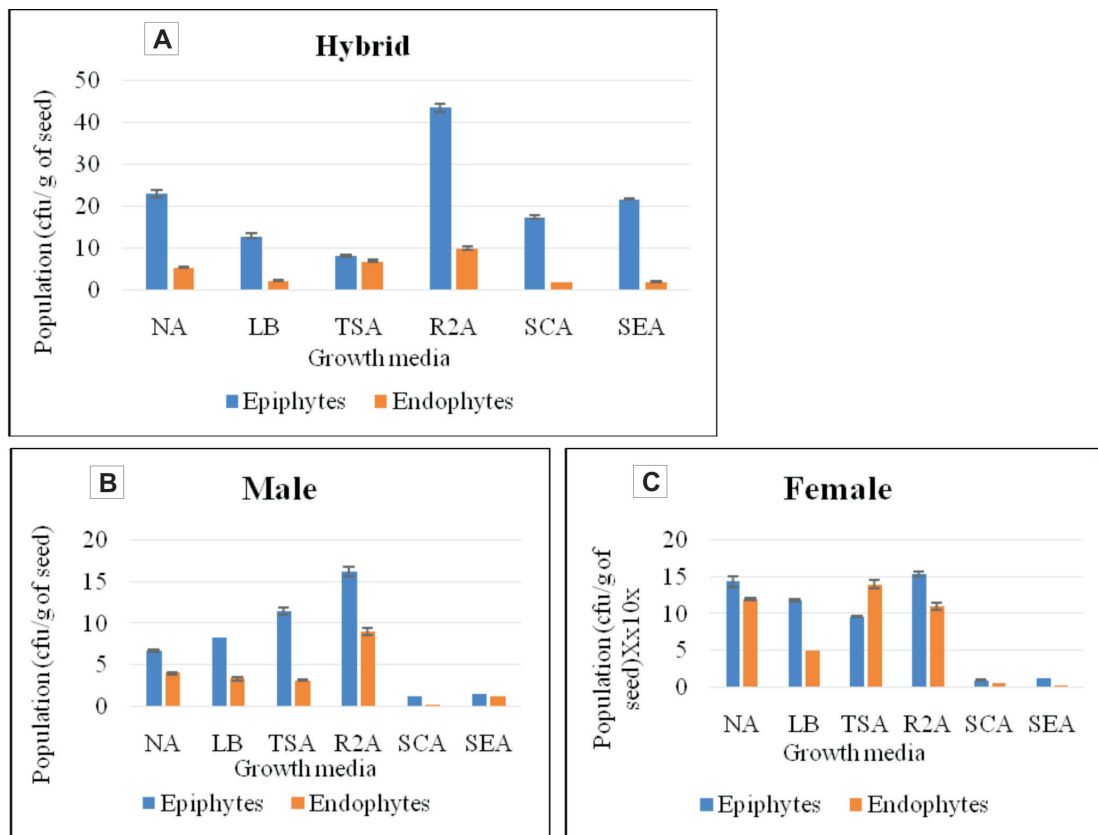


Fig. 1. Colony forming units of bacterial isolates of A) Male seeds B) Female seeds and C) Hybrid seeds of Maize COH6

of viable population count and colony morphological characteristics of seed associated bacterial microflora. The colony morphology and population count of epiphytes and endophytes revealed the existence of similarity and differences among the three different genotypes of maize seed. The population count expressed in colony forming units (cfu) per gram of seed shows epiphytes of seed microflora became more and the population decreased gradually inside the seeds (endophytes) (Santamaria and Bayman 2005). Kharwar *et al.*, 2010 reported that the concentration of fungal species dominance was found higher in epiphytes than endophytes, as it appears with *Cladosporium cladosporioides*. On comparing the male, female and hybrid seed microflora, majority of colony morphology of male and female seed microflora seems similar to the hybrid seed microflora. Comparing the morphological characteristics, the colonies of hybrid seeds seem similar to that of male and female seeds and also difference in colony morphology of some bacterial colonies were found. From these results, there could be movement of microorganisms transgenerationally from parents to off-

springs. The microbial community transferred vertically through seed from parental line to the hybrid (Vujanovic *et al.*, 2019). It could result in restoring the seed associated microorganisms and flow of microbial community composition across generation.

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