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Full Length Research Paper

Effect of pH and moisture content on endophytic colonization of maize roots

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The effect of pH and moisture contents on microbial colonization of maize roots was studied. Roots of SWAN and TZSRY cultivars were subjected to different pH levels (3, 6.9 and 11) and moisture contents (MC) of (30, 70 and 100%) for 5 weeks. The highest bacterial population (2.36 - 3.70 × 10^9 cfu/g root) was observed at pH 11 with SWAN cultivar and the least at pH 6.9 with TZSRY cultivar (1.24 - 1.62 × 10^9 cfu/g root). The highest fungal count (2.6 - 10.4 × 10^4 cfu/g root) was obtained throughout the period of study, at pH 3 with TZSRY. Both the bacterial and fungal populations were significantly different at the pH levels, with consistently higher count for pH 11 and 3, respectively. All the MCs showed a general decrease in bacterial population at the second and fifth week; however, MC (70%) with TZSRY had the highest population (2.0 - 1.02 × 10^9 cfu/g root). MC (30%) with SWAN showed a consistent high fungal population throughout the study at 3.6 - 13.0 × 10^4 cfu/g root. SWAN cultivar generally showed more bacterial and fungal colonization than TZSRY. *Bacillus* sp., *Saccharomyces* sp., *Pseudomonas fluorescens, Bacillus subtilis, Staphylococcus epidermidis* and *Micrococcus roseus* were the common endophytic microorganisms of both maize cultivars. This work shows that there were differences in the bacterial and fungal populations (resistance/susceptibility to environmental factors) in the roots of maize. The cultivars also differed in tolerance to pH and moisture contents implying that plants have influence on the microorganisms in its own rhizosphere.

Key words: Bacteria, fungi, endophytes, colonization, maize roots.

INTRODUCTION

Endophytes form mutual relationship with the plant harboring them while colonizing their hosts (Wilson, 1993; Saikkonen et al., 2004). Endophytic microorganisms can colonize all parts of the plants: Roots, leaves, stems, fruits, as well as the seeds (Johri, 2006). Endophytic microorganisms have been extensively studied for their beneficial importance (Chanway, 1996), they are known to promote plants growth and induce resistance to infection, they synthesize antimicrobial compounds (Clay and Schardl, 2002; Arnold and Herre, 2003; Atmosukarto et al., 2005; Santos et al., 2003) and are sources of bioactive compounds (Rodrigues-Heerklotz et al., 2001).

Endophytes gain entrance into plant parts mainly through the root system and also through the flowering parts, stems, cotyledons, radicles, stomata or wounds (Ajcann, 2007). Colonization of maize plants by endophytic microorganisms in particular (bacteria of the genus *Cellulomonas*, *Clavibacter*, *Curtobacterium*, *Microbacterium* and fungi *Acremonium zeae*) has been reported by Zinniel et al. (2002) and Poling et al. (2008), while the population and distribution of bacteria (*Burkholderia cepacia*) (Miller et al., 1989; Di Cello et al., 1997) and fungi (*Fusarium* sp., Vessiculo Arbuscular mycorrhizal fungi: genus *Scutellospora* sp., *Glomus* sp.) from the rhizosphere and roots of maize have been highlighted (Soonthornpoct et al., 2000; Jansa et al., 2003; Yamanaka, 2003).

Recent studies by Ceja-Navarro et al. (2010) characterized soil bacterial communities in zero tillage systems of maize. The authors reported the groups including the Caldilineales, Chromatiales, Oscilla-toriales, Legionellales, Nitroso-monadales, unclassified ∂-Proteobacteria, Bacillales, Burkholderiales, Pseudomonadales and

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Rubrobacteriales. The pH value of the soil is important as it affects the amount of minerals (Fe, Mg, Cu, Zn, Bo, HPO_4, Ca and Mg) available for plant usage (Anthony, 2003). The development of fungal diseases and transmission from host to host are encouraged at optimal pH values from 3 - 5 and at a temperature of 26°C (Anthony, 2003).

The effect of soil moisture on bacterial species could either be physiological or physical in nature. The soil moisture affects motility of bacteria, especially the chemotactic behavior (Bashan, 1999). Soil humidity and pH influence surface electrical charges between the soil particles and bacteria which determines the adsorption capacity of bacteria to soil particles. Reduced adsorption of bacteria to soil particles has been found to correlate with decreased soil water content or increased soil pH (Bashan, 1999). Oliveira et al. (2004) reported the importance of moisture in the sustenance of bacteria for a long period of time in the soil and that the detection of bacteria may be difficult at a low soil moisture level.

The objective of the study was to determine the pH and moisture content at which bacterial and fungal communities thrive best in maize roots.

MATERIALS AND METHODS

Collection of maize cultivars and preparation

The maize varieties used include the early season, yellow open pollinated, streak resistant variety - Tropical Zeal Smut Resistant Yellow (TZSRY-1), the Downy mildew resistant maize and the South West 1 (SWAN 1) cultivar. They were collected from the Agricultural Development Project (ADP) office in Ikare. The maize seeds were surfaced and sterilized with 0.8% NaOCl for 2 min followed by a 30 s dip in 70% ethanol and two rinses in distilled water according to the methods of Dietmar et al. (2008) and Zinniel et al. (2002) before planting.

Collection and preparation of soil samples

The soil used for this study was collected from a farm site in Akungba-Akoko, Ondo state at a depth of 15 cm. They were homogenized and autoclaved. Endophyte-free maize seedlings were raised according to the methods of Mejia et al. (2008) and Orole and Adejumo (2009). For each experiment, 5 seeds were initially planted to a pot, but later thinned to 2 most vigorous stands per pot in four replications making a total of 24 pots per experiment. Weed was controlled by hand throughout the duration of the experiments.

Preparation of pH levels

Solutions of Nitric acid (HNO3) and Ammonium hydroxide (NH4OH) were diluted and tested with pH meter until the pH values of 3 and 11 were respectively obtained. The above solutions together with distilled water (pH 6.9, served as control) were applied daily unto the pots planted with maize seeds until the 4th day after sowing, when the seeds germinated. Each pot was later watered on a daily basis with buffer solutions of Citric acid/Sodium citrate (pH 3), Sodium bicarbonate/Sodium hydroxide (pH 11) and distilled water respectively, till the termination of the experiment.

Measurement of soil moisture content

The soil moisture content was determined using the standard methods of Black (1965).

$$\text{Moisture content} = \frac{(\text{weight of wet soil} + \text{tare}) - (\text{weight of dry soil} + \text{tare})}{(\text{Weight of dry soil} + \text{tare}) - \text{Tare}}$$

Weight of water = weight of wet soil - weight of dry soil

Three moisture levels were tested: 30, 70 and 100%. Moisture was adjusted, with distilled water added to the pots to get the desired soil moisture content level.

Isolation of endophytic bacteria and fungi

The sampling times were 7, 14, 21, 28 and 35 days (5 weeks) after planting. Maize seedlings were uprooted and the roots severed 3 cm above the soil (Narisawa et al., 2003). They were properly labeled and brought to the laboratory. The roots were washed with distilled water and the surface was sterilized for 2 min with 70% ethanol and 2 min with 0.53% NaOCl (Mejia et al., 2008). They were rinsed in distilled water and dried afterwards (Ching-Hong and David, 2000; Zinniel et al., 2002). One gramme of the root was weighed, macerated with a sterile mortal and pestle. The root tissue extract was serially diluted in saline solution (NaOH) at 0.85% (Posada and Vega, 2005). Dilutions of 10^{-3} were made for fungal and 10^{-7} for bacterial isolation from which 1.0 ml of each sample was placed unto Petri dishes using the poured plate technique.

The culture medium used for fungi was Potato Dextrose Agar (PDA) (39 g/L of distilled water, Lab M Limited, Lancashire BL9 6AS, United Kingdom) in which Streptomycin 1.00 g/L was added to inhibit bacterial growth. For bacteria, Nutrient Agar (NA) (28g/L of distilled water, Sigma-Aldrich GmbH, CH-9471 Buchs, Switzerland) was used. The Petri dishes were incubated at 28°C (fungi) and 27°C (bacteria) for 72 h according to the methods of Gaviria (1978) and Zinniel et al. (2002) and were then examined.

Characterization of bacterial and fungal isolates

Colonies of fungal isolates were characterized between 48 - 96 h after inoculation. They were classified based on colony types and morphology of the spores on fungi according to the descriptions of various identification books and pamphlets including Dayan (2004).

Cultural characteristics like: opacity, elevation, edge and color were observed and recorded for the plates. Biochemical tests: Gram staining, motility, catalase and coagulase tests, sugar fermentation and MR-VP test (Methyl Red, Voges-Proskauer reaction) were done and additional characteristics described by Balows et al. (1992) and Bergey's Manual of Systematic Bacteriology (Krieg et al., 1984) were used for identification of the isolates.

Statistical analysis

A randomized complete block design (RCBD) was used for the experiments with the pots arranged in a split plot on the screenhouse benches. The 2 maize varieties were the main plots, while the 3 levels of each of the pH and moisture contents were subplots in 4 replicates. Statistical analyses were performed using Statistix 8.1 Analytical Software. Analysis of Variance (ANOVA) was performed and the treatment means were compared using Tukey

Table 1. Fungal population of maize roots at different pH levels and cultivars.

pH	Variety	Week 1	Week 2	Week 3	Week 4	Week 5
		Mean (cfu/g × 103 g root)				
3.0	SWAN	26.6ab[1]	68.0a	64.0a	87.0a	104.0a
3.0	TZSRY	17.0ab	30.0b	39.0bc	50.0b	73.0ab
6.9	SWAN	28.6a	34.0b	61.0ab	55.0b	48.0bc
6.9	TZSRY	18.6ab	24.0b	32.0c	42.0b	47.0c
11.0	SWAN	18.6ab	28.0b	18.0cd	21.0c	24.0c
11.0	TZSRY	15.0b	37.0b	16.0d	13.0c	24.0c
	Std error	3.2	4.9	3.0	4.9	6.9

ab[1]= Means with different letters are significantly different at p = 0.05 (Tukey HSD all-pairwise comparisons test).
Std error = Standard error for comparison of means.

Table 2. Fungal population in maize roots at different pH levels.

pH	Week 1	Week 2	Week 3	Week 4	Week 5
	Mean (cfu/g × 10^3g root)				
3.0	21.8ab[1]	49.0a	51.5a	68.5a	88.5a
6.9	23.7a	29.0b	46.5a	48.5b	47.5b
11.0	16.8b	32.5b	17.0b	17.0c	24.0c
Std error	2.3	3.4	2.1	3.4	4.8

ab[1]= Means with different letters are significantly different at p = 0.05 (Tukey HSD all-pairwise comparisons test).
Std error = Standard error for comparison of means.

Table 3. Bacterial population of maize roots at different pH levels and cultivars.

pH	Variety	Week 1	Week 2	Week 3	Week 4	Week 5
		Mean (cfu/g × 10^7 g root)				
3.0	SWAN	249.0a[1]	260.0a	176.0c	264.0ab	240.0bc
3.0	TZSRY	244.0ab	160.0c	84.0d	152.0c	160.0c
6.9	SWAN	190.0abc	228.0b	256.0b	344.0a	392.0a
6.9	TZSRY	124.0c	79.6e	100.0d	158.0bc	162.0c
11.0	SWAN	236.0ab	172.0bc	360.0a	370.0a	370.0a
11.0	TZSRY	172.0bc	110.0d	144.0c	296.0a	320.0ab
	Std error	24.5	8.02	9.5	30.9	31

a[1]= Means with different letters are significantly different at p = 0.05 (Tukey HSD all-pairwise comparisons test).
Std Error = Standard error for comparison of means.

HSD all-pairwise comparisons Test at p = 0.05 level of significance.

RESULTS

The maize cultivar, SWAN, had the highest fungal colonization of 10.4 × 10^4 cfu/g root at week 5 (pH 3) after germination, while colonization of 1.3 × 10^4 cfu/g root was obtained for TZSRY at week 4 (Table 1). Cultivar SWAN was better colonized by fungi (1.8 × 10^4 - 10.4 × 10^4 cfu/g root) than TZSRY at 1.3 × 10^4 - 7.3 × 10^4. At pH 3, fungal colonization increased steadily reaching a peak at the 5th week (8.8 × 10^4 cfu/g root) compared to the control (4.8 × 10^4 cfu/g root) and 2.4 × 10^4 cfu/g root at pH 11 (Table 2).

In the 2nd week (Table 3), TZSRY had the lowest bacterial colonization of 7.9 × 10^8 cfu/g root and the highest colonization at week 5 (3.2 × 10^8 cfu/g root), while SWAN obtained the lowest bacterial colonization of the roots at week 3 (1.7 × 10^8 cfu/g root) and the highest at week 5 (3.9 × 10^9 cfu/g root).

Bacteria colonization was highest at pH 11 (3.4 × 10^9 cfu/g

Table 4. Bacterial population in maize roots at different pH levels.

pH	Week 1	Week 2	Week 3	Week 4	Week 5
	Mean (cfu/g × 10^7g root)				
3.0	246.5 a[1]	210.0 a	130.0 c	333.0 a	200.0 c
6.9	157.0 b	153.8 b	178.0 b	251.0 b	277.0 b
11.0	204.0 ab	199.3 a	252.0 a	208.0 b	345 a
Std error	17.3	5.6	6.7	21.8	21.9

a[1]= Means with different letters are significantly different at p = 0.05 (Tukey HSD all-pairwise comparisons test).
Std Error = Standard error for comparison of means.

Table 5. Fungal population of maize roots at different moisture contents and cultivars.

MC (%)	Variety	Week 1	Week 2	Week 3	Week 4	Week 5
		Mean (cfu/g × 10^3g root)				
30	SWAN	36.0a[1]	42.0a	72.0a	107.0a	130.0a
30	TZSRY	13.0a	9.0 c	12.0b	17.0bc	24.0bc
70	SWAN	26.0a	28.0ab	44.0ab	57.0b	76.0b
70	TZSRY	20.0a	10.0bc	18.0b	18.0bc	24.0bc
100	SWAN	27.0a	16.0bc	15.0b	6.0c	5.0c
100	TZSRY	14.0a	23.0bc	9.0b	9.0c	8.0bc
	Std error	3.5	5.9	8.2	7.7	7.2

ab[1]= Means with different letters are significantly different at p = 0.05 (Tukey HSD all-pairwise comparisons test).
Std Error = Standard error for comparison of means.

Table 6. Fungal population of endophytes of maize roots at different moisture contents (MC).

MC (%)	Week 1	Week 2	Week 3	Week 4	Week 5
	Mean (cfu/g × 10^3g root)				
30	24.5a[1]	25.5a	42.0a	62.0a	77.0a
70	23.0a	19.0a	31.0a	37.5b	50.0b
100	20.5a	19.5a	12.0b	7.5 c	6.5c
Std error	2.5	4.1	5.8	5.4	5.1

ab[1]= Means with different letters are significantly different at p = 0.05 (Tukey HSD all-pairwise comparisons test)
Std error = Standard error for comparison of means.

root) when compared with the control (2.7 × 10^9 cfu/g root) and 2.4 × 10^9 cfu/g root of pH 3 (Table 4).

Cultivar SWAN at moisture content (30%) had the highest fungal colonization of 13.0 × 10^4 cfu/g root at week 5 as against 2.4 × 10^4 cfu/g root for TZSRY (Table 5). SWAN was better colonized by fungi at the three moisture content levels tested and a steady increase was observed (2.6 × 10^4 - 13.0 × 10^4 cfu/g root), while a decreasing colonization of 1.3 × 10^4 - 0.8 × 10^2 cfu/g root was obtained for TZSRY. At moisture content (30%), fungal colonization on the average increased along the week from 2.5 × 10^4 - 7.7 × 10^4 cfu/g root and at 70% MC, it was 2.3 × 10^4 - 5.0 × 10^4 cfu/g root, while the opposite was the situation at 100% MC where fungal colonization reduced from 2.0 ×10^4 cfu/g root in the 1st week to 0.7 × 10^4 cfu/g root in the 5th week (Table 6). MC (70%) with TZSRY had the highest population (2.0 - 1.02 × 10^9 cfu/g root) (Table 7), while all the MCs showed a general decrease in bacterial population at the second and fifth week. Consequently, the highest range of 2.5 - 19.8 × 10^8 cfu/g root was observed for 30% MC (Table 8).

The results on isolation of microorganisms from maize roots indicate that a total of 10 bacterial and 14 fungal species were obtained for SWAN and TZSRY, out of which 9 bacterial and 10 fungal species were isolated from the former, while 8 bacteria and 13 fungi from the latter, respectively. The most commonly observed fungus in all the samples was *Saccharomyces* sp. which was absent in pots treated with pH 11 of TZSRY. *Chaetomium globosum* and *Fusarium* sp. were isolated from the samples

Table 7. Bacterial population of maize roots at different moisture contents and cultivars.

MC (%)	Variety	Week 1	Week 2	Week 3	Week 4	Week 5
		Mean (cfu/g × 10^7g root)				
30	SWAN	200.0a[1]	33.0bc	60.0ab	200.0a	126.0bc
30	TZSRY	128.0b	17.0c	30.0b	196.0a	200.0a
70	SWAN	140.0b	52.0abc	16.0b	184.0a	188.0ab
70	TZSRY	200.0a	70.0ab	156.0a	152.0a	102.0c
100	SWAN	50.0c	70.0a	58.0ab	200.0a	180.0ab
100	TZSRY	136.0b	58.0ab	5.0b	154.0a	92.0c
	Std Error	12.2	9.8	23.1	26.2	17.3

a[1]= Means with different letters are significantly different at p = 0.05 (Tukey HSD all-pairwise comparisons test).
Std error = Standard error for comparison of means.

Table 8. Bacterial population of endophytes of maize roots at different moisture contents (MC).

	Week 1	Week 2	Week 3	Week 4	Week 5
MC (%)	Mean (cfu/g × 10^7g root)				
30	164.0a[1]	25.0b	45.0ab	198.0a	163.0a
70	170.0a	61.0a	86.0a	168.0a	145a
100	93.0b	64.0a	31.5b	177.0a	136a
Std error	8.6	6.9	16.3	18.5	12.2

ab[1]= Means with different letters are significantly different at P=0.05 (Tukey HSD all-pairwise comparisons test).
Std error = Standard error for comparison of means.

samples with 30% moisture content of TZSRY, while *Phoma* sp. was present only in the samples with pH 3 of TZSRY. Interestingly, *Pseudomonas fluorescens, Bacillus subtilis* and *Micrococcus* sp. were observed in all the samples.

DISCUSSION

Normal soil contains enormous number of microbes and substantial quantities of microbial biomass, and generally, soil microbes grow best in soils with close neutral pH (pH 6.0 - 8.0) having adequate supplies of inorganic nutrients (nitrogen, phosphorus, potassium, sulphur, other elements and trace metals), aeration (a balance of air and water-filled pore space (about 50 - 60% of water holding capacity)), abundant organic substrates (carbon and energy sources from crop residues, organic wastes) and temperature (10 - 40°C) (Ventura, 2000; Haney et al., 2000).

In this investigation, bacterial colonization was highest at pH 11. This is not surprising, since bacteria grow in slightly alkaline medium (alkaliphiles), although some bacteria can grow at high pH only, some at low pH, some have a broad pH range and others a narrow range. For most bacteria, there is an orderly increase in growth rate between the minimum and the optimum and a corresponding orderly decrease in growth rate between the optimum and the maximum pH, reflecting the general effect of change [H^+] on the rates of enzymatic reaction. There are neutrophiles, acidophiles and alkalinophiles based on the pH of the habitat of an organism and any change affects the population, because strong acids and bases can be highly damaging to enzymes and other cellular substances (Brock, 1986; Talaro, 2005).

It was observed that both bacteria and fungi grew at all the pH levels and moisture contents tested. This agrees with the observation of Erland et al. (1990) that mycorrhizal fungi possess a generally broader range of pH tolerance in symbiosis than in pure culture and emphasized the danger of extrapolating the results from pure culture studies to symbiotic systems, while Yamanaka (2003) highlighted that many of the saprotrophic fungal species grew well at pH 7 or 8. The ectomycorrhizal species showed optimum growth at pH 5 or 6. High pH stresses and eliminates fungi, especially those causing root rots (*Fusarium verticillioides* and *F. avenaceum* causing seedling root rot and *Acremonium strictum* causing black bundle disease and late wilt) and makes the bacteria and actinomycetes to dominate. Among the *Fusarium* sp. known to colonize maize roots, *F. verticillioides*, *F. oxysporum*, *F. proliferatum* and *F. solani* were considered as "rhizosphere competent" (Ocamb and Kommedahl, 1994) because they grow saprophytically, reproduce in the rhizosphere and cause root rot when host plants are under stress (Young and

Kucharek, 1977). Differences in colonization may also be due to changes in both the nutrient content of the soil and microbial activity (Jansa et al., 2003).

The cultivar SWAN had a higher bacterial colonization compared to cultivar TZSRY. SWAN is a hybrid maize developed for the South Western Region of Nigeria; Downy mildew resistant; and was meant to survive the Guinea Savanna. TZSRY however, is an early season, yellow, open pollinated and streak resistant. These differences in the cultivars account for their microbial tolerance and susceptibility. Soil pH can drop below 5.0 after prolonged use of ammonia-based fertilizers or acid rain and this can cause marked reductions in populations of bacteria and actinomycetes and simultaneous increases in the relative abundance of fungi in the field (Ventura, 2000; Haney et al., 2000). These changes are easily reversed with applications of lime to the soil.

Proper moisture conditions are important for microbial growth. Water must be able to flow freely in and out of cells for transfer of nutrients and waste products. The result of the bacterial colonization, when moisture content varied, is in agreement with the report of Oliveira et al. (2004) that bacteria in tropical areas have the capacity to survive varying conditions with ease. For example, *Azospirillium* species has been found to use varying mechanisms to survive during unfavorable environmental conditions including cyst formation, melanin production and flocculation which may explain its high incidence at high and low moisture contents in this experiment. Other factors that may affect the variation in colonization rate of the microorganisms include the rooting pattern and soil condition. Soil microbiota has been found to respond quickly to environmental changes and they therefore serve as efficient bioindicators of soil conditions (Avidano et al., 2005). Prevalence of soil-borne pathogens like *Fusarium* sp. in maize can cause severe diseases, thereby reducing plant vigor, growth and crop yields, while the abundance of beneficial root and soil organisms can suppress pathogens and diseases, improve plant nutrition, promote growth and increase productivity (Le´vesque and Rahe, 1992; Larkin, 2003).

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