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RHIZOSPHERIC BACTERIAL DIVERSITY: IS IT PARTLY RESPONSIBLE FOR WATER DEFICIENCY TOLERANCE IN WHEAT?

RUBINA ARSHAD*, SHAFQAT FAROOQ AND FAROOQ-E-AZAM

Nuclear Institute for Agriculture and Biology (NIAB), PO Box 128, Jhang Road, Faisalabad, Pakistan

Abstract

Bacterial diversity plays a key role in agricultural environments and is promising for its potential use in sustainable agriculture. The present study was conducted to assess the influence of moisture stress on bacterial diversity in the rhizoplane of wheat line WL-1076. Healthy seeds were grown in potted soil maintained at moisture contents equivalent to 133% (L1), 100% (L2), 80% (L3), and 55% (L4) of field capacity. Soil closely adhering to the roots of the plants was removed at heading, grain filling and maturity stage. For each soil sample, one gram of soil was taken for evaluating bacterial population by cultivation method. Moisture stress caused reduction in the bacterial population from $7.13x10^8$ cfu g^{-1} (control) to $1.03x10^8$ cfu g^{-1} (L4: moisture) suggesting that the imposed stress directly affects population dynamics of bacteria in rhizoplane. The population size in normal soil was higher at grain formation stage than at heading or maturity stage and the same trend observed in soil with different moisture stress levels. Moisture deficit changed markedly the structure of soil bacterial communities. Cluster analysis revealed that bacterial community in rhizoplane was represented by mixed population of nine species *Staphylococcus aureus* (30%), *Bacillus subtilis* (25%), *Enterobacter aerogenes* (11%), *Bacillus megaterium* (8%), *Klebsiella aerogenes* (8%), *Escherichia coli* (6%), *Kluyvera cryocrescens* (6%), *Providencia rettgeri* (3%) and *Proteus vulgaris* (3%). *Staphylococcus aureus* (30%) and *B. subtilis* (25%) were relatively abundant in rhizoplane and generally present at all growth stages and moisture levels, indicating their resistance to water stress. Bacterial diversity in rhizoplane (some of which are known to produce exo-polysaccharides) might have provided optimum moisture to the roots to keep them alive and could thus be partly responsible for water deficiency tolerance in wheat.
Key words: Bacterial diversity, rhizoplane, wheat, water stress

Introduction

Bacterial diversity plays a key role in agricultural environment and is promising for its potential use in sustainable agriculture. Soil microorganisms mediate many processes such as recycling of plant nutrients (Powlson *et al*., 2001), maintenance of soil structure (Young *et al*., 1998), degradation of agro-chemicals and pollutants (Sigler *et al*., 2003) and the control of plant and animal pests (Parkinson & Coleman, 1991). According to an estimate, 80-90% of the processes in soil are mediated by microbes (Coleman & Crossley, 1996; Nannipieri *et al*., 2003). Although the relationship between soil microbial diversity and the functioning and sustainability of agricultural ecosystems is unclear, studies have been documented (Swift & Anderson, 1993; Fragosa *et al*., 1997), which showed the importance of bacterial diversity in key functions of agro-ecosystems such as influence on ecosystems by contributing to plant nutrition (George *et al*., 1995; Timonen *et al*., 1996), plant health (Srivastava *et al*., 1996; Filion *et al*., 1999; Smith & Goodman,

* Corresponding Author: Rubina Arshad; arshadrubina@hotmail.com; Fax: 0092 41 2654213

1999), soil structure (Wright & Upadhya, 1998; Dodd *et al*., 2000) and soil fertility (Yao *et al*., 2000; O'Donnell *et al*., 2001). The present study was based on bacterial diversity detected in the rhizoplane of water deficiency tolerant wheat (Farooq & Azam, 2006). The objectives were to characterize bacterial diversity in the rhizoplane of wheat exposed to different moisture stresses, and to assess the influence of moisture stress on bacterial diversity and its possible relationship with water deficiency tolerance in wheat.

Materials and Methods

Soil samples: Seeds of wheat (*Triticum aestivum* L.) line WL-1076 were sown in plastic pots each containing 10 kg silty clay soil. After 15 days of germination, different levels of moisture equivalent to 133% (L1: normal irrigation given to wheat), 100% (L2), 80% (L3), and 55% (L4) of field capacity were applied and maintained by providing irrigation after a regular interval of four days. Soil closely adhering to the roots of the test plants was removed at heading, grain formation or maturity stages of growth and processed for studying bacterial diversity. Each sample was analyzed immediately after it reached to the laboratory.

Cultivation conditions: Bacterial population was detected by cultivation method using two media nutrient agar (NA) and peptone-yeast extract-dextrose agar (PYDA). For each sample, one gram of soil was mixed with 10 ml of sterile distilled water for 2-3 minutes and then serially diluted up to 10^{-7}. One ml of each of the diluted soil and water sample was transferred into a test tube. Approximately 9 ml of sterile water was added to each tube and mixed thoroughly. Spread plate method was used to count bacterial colonies. Suitable dilution (10^{-5}, 10^{-6}, and 10^{-7}) for each sample was plated by transferring 0.1ml to nutrient agar plate. Plates were incubated at 37°C for 18-24 h. After incubation various bacterial colonies were examined and calculated through a colony counter. The count per g of soil was calculated as follows:-

$$\text{Organisms per g soil} = \frac{\text{Number of colonies}}{\text{Volume of sample plated x dilution}}$$

Characterization of colonies: Individual colonies were characterized on the basis of colony morphology (shape, size, texture and color), Gram-staining and conventional biochemical tests. Streak plate method was used to obtain single and pure colony. Each isolated colony was streaked on nutrient agar slants and incubated at 35-37ºC for up to 48 h to obtain optimum growth. Colony morphology was determined after 2 to 3 days of growth on nutrient agar plates incubated at 35-37ºC. Each isolate was subjected to Gram staining (Baker, 1962) and examined for cellular morphology. The biochemical tests were performed according to Holt *et al*. (1994). All the strains were subjected to methyl red, Voges-Proskauer, nitrate reduction; fermentation and oxidation, citrate utilization, acid production in phenol red broth base containing 1% carbohydrate source (starch, fructose, glycerol, glucose, lactose, maltose and sucrose) and indole production tests. Purified isolates were maintained on nutrient agar slants.

Statistical analysis: Cluster analysis was used for evaluating the relative abundance (evenness) and diversity of bacterial species (richness) in different moisture stress levels

and growth stages. Hierarchical cluster analysis was made by the complete linkage method and with the Euclidean distance measure. Dendograms of bacterial population based on the relative abundance and richness were constructed. “Statistica” software was used for computing the data.

Results

Bacterial Population in the rhizoplane of wheat: Moisture stress reduced the bacterial population from $7.13 \pm 0.21 \times 10^8$ cfu g^{-1} (control) to $1.03 \pm 0.06 \times 10^8$ cfu g^{-1} (L4). The effect of moisture stress at L4 (the lowest moisture level) was most pronounced for the bacterial count ($1.03 \pm 0.06 \times 10^8$ cfu g^{-1}) at heading stage (Table 1). The cultivable population size in normal soil was higher ($7.13 \pm 0.21 \times 10^8$ cfu g^{-1}) at grain formation stage than at heading ($6.41 \pm 0.41 \times 10^8$ cfu g^{-1}) or maturity ($4.13 \pm 0.15 \times 10^8$ cfu g^{-1}) stages and the same trend was observed in soil with different moisture stress levels.

Table 1. Bacterial density in the rhizoplane of wheat grown under moisture stress at different growth stages.

Growth Stage	Total cell count (10^8) per g of soil (cfu g^{-1})			
	Moisture levels (Mean ± SD)			
	133%* (L1)	100% (L2)	80% (L3)	55% (L4)
Heading	6.41 ± 0.41	5.46 ± 0.35	3.91 ± 0.36	1.03 ± 0.06
Grain Formation	7.13 ± 0.21	6.52 ± 0.40	5.23 ± 0.35	3.16 ± 0.31
Maturity	4.13 ± 0.15	3.23 ± 0.32	2.74 ± 0.21	2.04 ± 0.32

* Percent of field capacity

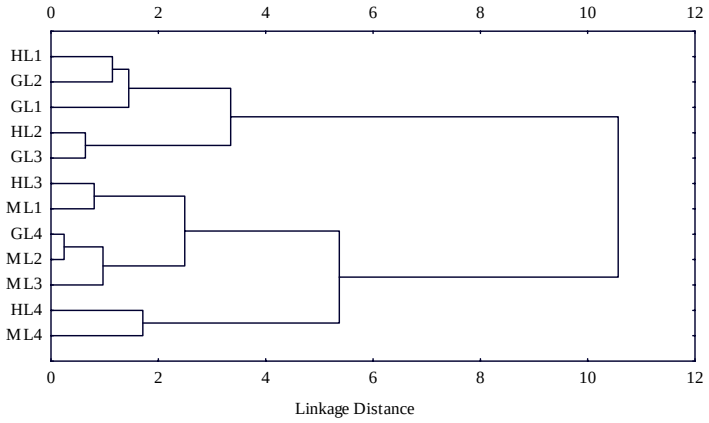

Fig. 1. Distribution of bacterial population in the rhizoplane of wheat grown under varied moisture levels (H: Heading; G: Grain Formation; M: Maturity; L1=133%; L2=100%; L3= 80%; L4=55%).

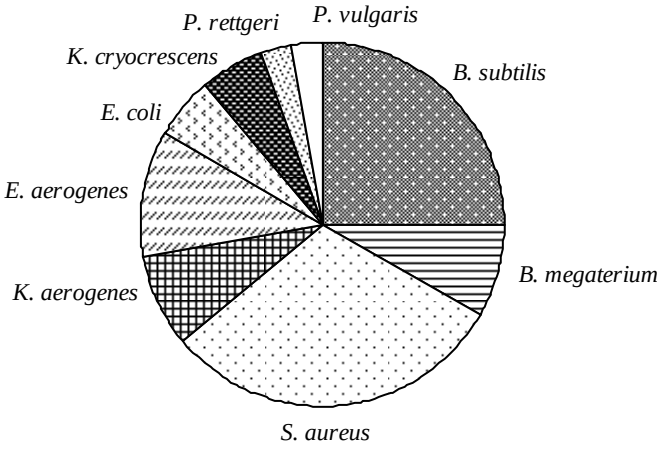

Fig. 2. Percent distribution of bacterial species obtained from the rhizoplane of wheat grown under moisture stress.

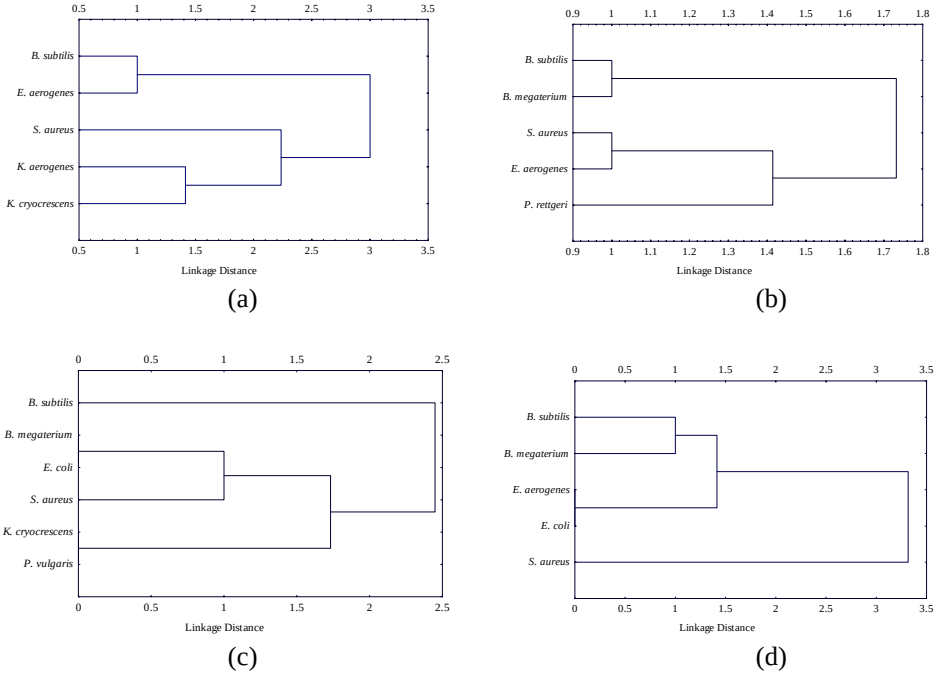

Fig. 3. Distribution of the dominant bacterial species in the rhizoplane of wheat at different moisture stress levels; (a): 133%, (b): 100%, (c): 80%, (d): 55% field capacity.

Relative abundance of species: Hierarchical clustering resulted into three distinct groups of abundance according to similar bacterial population (Fig. 1). One clustered at heading (L1 and L2) and grain formation stages (L1, L2 and L3) with relatively more bacterial abundance. The second group with relatively low bacterial population clustered at heading (L3, L4), maturity (L1, L2, L3, L4) and grain formation (L4) stages. The closest pair was formed by grain formation (L4) and maturity (L2) with a linkage distance of 0.24, followed by grain formation (L3) and heading (L2) at 0.64 and grain formation (L4) and maturity (L3) at 0.82 linkage distance. It confirmed the highest degree of similarity in the bacterial population existing at these closest moisture levels and growth stages. The farthest pair was formed between grain formation (L1) and heading (L4) stage with a linkage distance of 10.6 showing a link between maximum and minimum bacterial abundance at grain formation stage in normal soil and at heading stage with maximum moisture stress, respectively. Other closely linked growth stages and moisture stress levels exhibited the same pattern.

Diversity of species: On the basis of the phenotypic characteristics, bacterial isolates were classified into different groups (data not shown). The percent distribution of specific bacterial species is shown in Figure 2. Overall, nine bacterial species *Staphylococcus aureus* (30%), *Bacillus subtilis* (25%), *Enterobacter aerogenes* (11%), *Bacillus megaterium* (8%), *Klebsiella aerogenes* (8%), *Escherichia coli* (6%), *Kluyvera cryocrescens* (6%), *Providencia rettgeri* (3%) and *Proteus vulgaris* (3%) were identified at different growth stages. Among these species, *S. aureus* (30%) and *B. subtilis* (25%) were relatively abundant in rhizoplane. Six bacterial species (*K. aerogenes, K. cryocrescens, P. rettgeri, E. aerogenes, B. subtilis* and *S. aureus*) were identified at the heading stage and *S. aureus* was the most dominant species. At the grain formation stage, a total of seven species (*S. aureus, B. subtilis, K. aerogenes, E. aerogenes, E. coli, K. cryocrescens* and *P. vulgaris*) were found and among these, *S. aureus* and *B. subtilis* were equally dominant. Only four species, *B. subtilis, E. aerogenes, B. megaterium* and *S. aureus* were observed at the maturity stage with almost equal distribution.

Overall five different bacterial species were identified in normal soil (L1). The closest pair was formed by *B. subtilis* and *E. aerogenes* with a linkage distance of 1.0 and the farthest pair was formed between *B. subtilis* and *K. aerogenes* with a linkage distance of 3.0 showing the dominance of *K. aerogenes* over the other species in normal soil (Fig. 3a). At moisture stress with 100% field capacity (L2), a total of five species were found and *S. aureus* was the predominant species (Fig. 3b). At moisture level of 80% of the field capacity (L3), four species (*B. megaterium, E. coli, K. cryocrescens* and *P. vulgaris*) exhibited the same pattern of distribution (Fig. 3c). Out of the remaining two species (*B. subtilis* and *S. aureus*), *B. subtilis* was the most prevalent species. On the contrary, at moisture level equal to 50% of field capacity (L4), *S. aureus* out-numbered the *B. subtilis* with a linkage distance of 3.16 (Fig. 3d). The remaining three species (*B. megaterium, E. coli* and *E. aerogenes*) were found in low proportion.

Discussion

Rhizosphere is a major soil ecological environment for plant microbe interactions involving colonization of different microorganisms in and around the roots of the growing plants. Most rhizosphere bacteria and fungi are highly dependent on associations

with plants that are clearly regulated by root exudates (Bais *et al*., 2004). The accurate characterization of bacterial populations naturally associated with the roots of plants growing under stressed conditions is therefore, very important. In the present study, we have observed a strong influence of various levels of moisture stresses on bacterial diversity and suggested that water deficit directly affects population dynamics in the rhizoplane of wheat line WL-1076. Adverse effects of water stress on plant height, dry matter yield and leaf area index of wheat (Peschke *et al*., 1997) and the effect of water stress upon the diversity and cultural activity of bacterial communities in the rhizosphere of an established upland grassland soil have been reported (Griffiths *et al*., 2003). Although previous studies indicated that the composition and diversity of soil bacterial communities can be influenced by a wide range of biotic and abiotic factors (Buckley & Schmidt, 2002) and soil moisture is an important factor which can directly affects the physiological status of bacteria (Harris, 1981), but no information is so far available on the exact characterization of bacterial diversity in the rhizoplane of wheat growing under various levels of moisture stresses. The present study is first of its kind. Since moisture regulates substrate availability, diffusion of gases, soil pH, temperature and osmotic status of bacteria therefore, it can directly exert a strong selective pressure on bacterial communities associated with rhizoplane. Also, since the plant themselves are directly influenced by the soil moisture, it can in turn also indirectly affect bacterial communities through changes in rhizodeposition and nutrient allocation below ground (Lynch & Whipps, 1990).

The comparisons of the three elements of diversity in a sample *i.e*., the types of bacteria present (composition), the number of types (richness), and the frequency distribution of relative abundance of types (structure) can be made by clustering of isolates into operational taxonomic units based on phenotypic or genotypic characteristics (Dunbar *et al*., 1999). In the present study, cluster analysis was used for evaluating the relative abundance and diversity of bacterial species in different moisture stress levels and growth stages. The data obtained clearly demonstrated treatment effects (growth stages/moisture levels) on relative bacterial abundance. It was observed that plant growth stage had a strong impact on total bacterial communities. The frequency of bacteria was very low at heading and very high at the grain formation stages and was probably due to the root excretions in the rhizosphere that diffuse in the soil. It is reported that 5% (Walker *et al*., 2003) to 60% (Marschner, 1995) of photosynthetic carbon fixed by the plant can be transferred to the rhizosphere by exudation through its root system. The relative abundance and high diversity at grain formation stage might have been due to the fact that in the older root zones, more carbon compounds such as cellulose and hemicellulose were deposited so that bacteria in these zones were presumably adapted under moisture stress. Also, since the composition of root exudates is strongly affected by the plant developmental stage therefore bacteria in the rhizoplane were significantly affected by growth stage. The root exudates at grain formation stage might have been liked by the bacteria therefore, their population increased at that stage.

In nature, plants have to face different stresses from seedling to maturity stages (Bernstein, 1975). Physiological stress could reduce total soil microbial diversity by favoring a portion of the microbial community best adapted to coping with the given stress (Schimel *et al*., 1999). In the present study, we found a mixed population of nine species (*S. aureus, B. subtilis, B. megaterium, K. aerogenes, E. aerogenes, E. coli, K. cryocrescens, P. rettgeri* and *P. vulgaris*) that colonized the rhizoplane of water stressed

wheat line and two of them: *S. aureus* (30%) and *B. subtilis* (25%) were present in all growth stages and moisture levels. As reported earlier by Griffiths *et al*. (2003), the imposed moisture stress modulates the physiological status of the bacterial community therefore the surviving fractions in the moisture stressed soils might have developed resistant to water stress. The moisture stress induces osmotic shock which can result into cell lyses and a release of intracellular solutes (Fierer *et al*., 2003). In the present study, this phenomenon might have eliminated different bacterial communities at different growth stages and stress levels which have resulted ultimately into nine representatives that might have survived rapid changes in water potential.

The presence of two species (*S. aureus* and *B.subtilis*) among the nine under all moisture stresses could have been due to the fact that both are Gram-positive bacteria which had thicker, more rigid cell walls and compatible solutes that enhance osmoregulatory capabilities. These bacteria can therefore, survive under extreme water deficient conditions and since they stay in the soil particles closely adhering to the roots and therefore, can provide optimum moisture to the roots to keep them alive. It is therefore, possible that these bacterial species are partly responsible for water deficiency tolerance in wheat line used in the present study. Although, more studies on diverse wheat genotypes are essential to draw any meaningful conclusion nevertheless, this is a new dimension to study water deficiency tolerance in crop plants for identification of genotypes to be grown under water deficient conditions.

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