THE INFLUENCE OF SOIL WATER POTENTIAL ON THE GROWTH AND SURVIVAL OF ALFALFA RHIZOBIA IN THE SOIL

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ABSTRACT

Growth and survival of alfalfa rhizobia (*Rhizobium meliloti* Dang.) from Pakistan and Nepal were studied in vials filled with sterile soil maintained at -0.03, -1.0, and -1.5 MPa. The main effects of water level, rhizobial strains and length of exposure to desiccation and their interactions showed a highly significant ($P \leq 0.001$) effect on the number of rhizobia g^{-1} of soil. Growth and survival of rhizobia were highest in soil at -0.03 MPa followed by soil at -1.0 and -1.5 MPa. Highest cell counts were observed for strain UL 136 followed by strain UL 222 and the lowest was for strain UL 61. Two rhizobial strains (UL 136 and UL 222) were most tolerant to desiccation and showed highest growth and survival under low water potential. These two strains probably could be used as inoculants for alfalfa production under arid and semiarid environments.

KEY WORDS: Alfalfa rhizobia, desiccation tolerance, inoculant production, soil water potential, sterile soil.

INTRODUCTION

The benefits from legume inoculation with *Rhizobium* depend on the survival of effective rhizobia in the soil and rhizosphere of the legume. The successful introduction of a rhizobial strain, and its persistence and relative nodulating ability in subsequent seasons are affected by a number of biotic and abiotic factors (Bordeleau and Prevost 1994). Both high and low soil water contents are potentially deleterious to rhizobia (Barnet et al. 1988). Drought may influence the symbiosis by affecting the growth and survival of rhizobia in the soil (Barnet et al. 1988, Bordeleau and Prevost 1994). Soil water content affects the number of rhizobia in the soil, their distribution through the soil profile, and the susceptibility of the plant root to infection (Sprent and Zahran 1988, Bordeleau and Prevost 1994).

Reduced viability of various rhizobial strains with drought has been reported for *R. meliloti* (Busse and Bottomley 1989, Mary et al. 1985, 1994; Mohammad et al. 1991), *R. trifolii* (Osa-Afiana and Alexander 1979, Fuhrmann et al. 1986), *R. leguminosarum* (Mahler and Wollum 1981, Leung and Bottomley 1994), *R. lupini* (Miller and Pepper 1988), and *Bradyrhizobium japonicum* (Mahler and Wollum 1981, Boon-

kerd and Weaver 1982, Mary et al. 1993). As a matter of fact, differences in tolerance against various desiccation stresses have been reported among strains within species of rhizobia (Mary et al. 1986, 1994), perhaps due to reduced metabolic activity at low water potentials (Wilson and Griffin 1975). However, Mugnier and Jung (1985) and Mary et al. (1994) have mentioned that *R. meliloti* is more tolerant to desiccation stress than *B. japonicum*.

Alfalfa (*Medicago sativa* L.) is one of the main legumes used for forage production in Pakistan and is grown on the irrigated plains and in rainfed areas (Michaud et al. 1988). In many areas of Pakistan, little or no rainfall occurs throughout much of the year and these prolonged dry seasons probably reduce rhizobial growth and survival. Specific data are not available concerning how alfalfa rhizobia from Pakistan respond to drought. This study was conducted to examine the influence of soil moisture potential on the growth and survival of alfalfa rhizobia from Pakistan and Nepal.

MATERIALS AND METHODS

Ten strains of alfalfa rhizobia (*R. meliloti*) from the Pakistan and Nepal collection were evaluated for their ability to grow and survive under drought. All the eight strains from Pakistan were isolated from alfalfa (*M. sativa*) naturally growing in the mountainous rangelands of Federally Administered Northern Areas (FANA). Two of the strains (UL 150 and UL 167) were isolated from yellow-flowered alfalfa

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(*M. falcata* L.) collected from Nepal. Detailed information about site, location and the origin of these strains is provided elsewhere (Athar and Johnson 1996). These strains were selected based on the results of a previous study that evaluated their competitiveness on three alfalfa accessions (Athar and Johnson 1996).

A total of 10 g of oven dry soil consisting of three parts sandy loam (Kidman series, coarse-loamy, mixed, mesic, Calcic Haploxeroll) to one part peat moss was placed in each 30 mL screw-capped vial. The soil-filled vials were sterilized by autoclaving for two hours on two successive days at 121°C. Soil in these vials was maintained at one of three water levels (-0.03, -1.0 and -1.5 MPa) based on a soil moisture release curve obtained with a pressure plate apparatus by the Plant, Soil, and Water Analysis Laboratory at Utah State University (Fig. 1). The three matric potentials were established by relating the gravimetric soil water content required for each desired water potential.

Fig. 1. Soil moisture release curve as determined by the pressure plate method.

Each vial was adjusted to one of the three water levels by adding a combination of inoculum consisting of 0.3 mL of single strain broth culture of one of ten strains of rhizobia and an amount of water to adjust the soil to the desired soil water potential. Inoculum was prepared by growing each strain in yeast extract-mannitol broth on a rotary shaker at 200 rpm at 28°C for 4 days. The experiment was conducted using a randomized complete block design with three replications. The vials were kept in the dark at 25°C. Rhizobia in the soil-filled vials were enumerated at 0, 4, 8, 14, 21, and 28 days after inoculation by preparing 10-fold serial dilutions and plating on yeast-mannitol agar medium as described by Somasegaran and Hoben (1994). The colonies developed in each plate were counted after incubating the plates for 3 days at 25°C. Data were analyzed statistically and means were compared with the LSD multiple mean comparison test. All significance levels are at $P \leq 0.05$ unless otherwise stated.

RESULTS

The main effects of time, rhizobial strains, and water level had a highly significant ($P \leq 0.001$) effect on the number of rhizobia g^{-1} of soil. Highest cell counts were observed at -0.03 MPa followed by soil maintained at -1.0 and -1.5 MPa. The ten rhizobial strains differed significantly in cell counts. Rhizo-

bial population decreased significantly with time. A marked loss of viable cells was observed during the first two weeks of the experiment with a more gradual decline thereafter.

A significant ($P \leq 0.001$) interaction between length of exposure to drought and water potential was observed for number of rhizobia g^{-1} of soil. The cell count was highest at the beginning of the experiment and declined with time until the last enumeration at 28 days (Table 1). The rhizobial population declined 4% in soil at -0.03 MPa and 13% at -1.0 and -1.5 MPa after 28 days. Rhizobia enumerated after 4 days at water potential -0.03 MPa did not show any significant difference from the number of rhizobia counted on the day of inoculation. However, rhizobia showed a gradual decrease at the four later dates and were significantly different from each other. At -1.0 and -1.5 MPa, significant declines in numbers of rhizobia were observed at each sampling date.

TABLE 1. Effect of soil water potential on the population growth and survival of *Rhizobium meliloti* averaged across strains.

Time (Days)	Water level (MPa)		
	-0.03	-1.00	-1.50
(Log number of rhizobia g^{-1} soil)			
0	8.26 e	8.15 f	8.09 f
4	8.26 e	7.98 e	7.92 e
8	8.21 d	7.71 d	7.66 d
14	8.15 c	7.32 c	7.27 c
21	8.05 b	7.16 b	7.11 b
28	7.96 a	7.06 a	7.02 a

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

A significant ($P \leq 0.001$) interaction was found between rhizobial strains and soil water potential. All the rhizobial strains showed highest growth in soil maintained at -0.03 MPa (Table 2). A significant decrease in cell counts was observed for all rhizobial strains except strain UL 222 as drought increased from -0.03 to -1.0 and -1.5 MPa. Cell

TABLE 2. Effect of soil water potential on the population growth of *Rhizobium meliloti* averaged across sampling dates.

Strains	Water level (MPa)		
	-0.03	-1.00	-1.50
(Log number of rhizobia g^{-1} soil)			
UL 61	8.12 ab	7.33 a	7.29 a
UL 115	8.12 ab	7.62 c	7.53 c
UL 129	8.12 ab	7.62 c	7.55 c
UL 136	8.25 e	8.06 e	8.01 e
UL 150	8.15 bc	7.36 ab	7.31 ab
UL 167	8.19 d	7.36 ab	7.32 ab
UL 201	8.17 cd	7.38 ab	7.33 b
UL 207	8.15 bc	7.36 ab	7.31 ab
UL 210	8.10 a	7.60 c	7.55 c
UL 222	8.09 a	7.93 d	7.92 d

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$. Within each row, values between each column also differed significantly at $P \leq 0.05$ for all the strains except UL 222, which did not differ between -1.0 and -1.5 MPa.

TABLE 3. Survival of *Rhizobium meliloti* strains averaged across 3 water levels.

Strain	Time (Days)					
	0	4	8	14	21	28
	(Log number of rhizobia g ⁻¹ soil)					
UL 61	8.123 a	8.066 c	7.736 a	7.273 a	7.180 a	7.095 a
UL 115	8.121 a	7.828 a	7.811 b	7.775 c	7.551 c	7.442 d
UL 129	8.255 bc	7.877 b	7.830 b	7.755 bc	7.480 b	7.374 c
UL 136	8.540 c	8.210 e	8.129 c	8.057 d	7.911 d	7.785 e
UL 150	8.216 b	8.078 cd	7.765 a	7.320 b	7.173 a	7.100 a
UL 167	8.245 bc	8.110 cd	7.755 a	7.260 a	7.201 a	7.158 ab
UL 201	8.240 bc	8.115 d	7.763 a	7.306 ab	7.208 a	7.138 ab
UL 207	8.240 bc	8.082 cd	7.764 a	7.257 a	7.193 a	7.124 a
UL 210	8.193 b	7.869 b	7.834 b	7.724 bc	7.488 b	7.400 cd
UL 222	8.521 c	8.270 f	8.201 d	8.056 d	8.013 e	7.828 e

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

counts of strain UL 222 did not differ between -1.0 and -1.5 MPa (data not shown). The two strains that produced the highest growth at -1.0 and -1.5 MPa were UL 136 and UL 222. Strain UL 136 exhibited greater growth than UL 222 at -1.0 and -1.5 MPa. Five of the strains (UL 61, UL 150, UL 167, UL 201, and UL 207) exhibited significantly lower growth at -1.0 and -1.5 MPa than the other strains.

There was a significant ($P \leq 0.001$) interaction between length of exposure to drought and rhizobial strains. The growth of all ten rhizobial strains was highest at the beginning of the experiment and decreased significantly with time (Table 3). Strains UL 136 and UL 222 had a significantly higher survival than the other eight rhizobial strains after 28 days. Five of the strains (UL 61, UL 150, UL 167, UL 201, and UL 207) showed low survival after 28 days and did not differ among themselves. The influence of soil moisture potential -1.5 MPa on the growth and survival of each individual strain is presented in Table 4. The cell counts decreased significantly ($P \leq 0.05$) over time for all strains. However, two of the strains (UL 136 and UL 222) showed higher survival than the other strains at soil moisture potential -1.5 MPa.

DISCUSSION

Soil water potential affected the population growth and survival of all ten strains of rhizobia. All rhizobial strains showed greatest growth at -0.03 MPa and significant reductions with increasing drought. These results agree with those of others, who also reported decreased rhizobial growth at low water potentials (Osa-Afiana and Alexander 1979, Mahler and Wollum 1981, Griffith and Roughley 1992, Leung and Bottomley 1994, Mary et al. 1994). Strains of rhizobia have been shown to vary in their tolerance to decreasing water potential (Wilson and Griffin, 1975). Poor rhizobial growth observed at low water potentials could be due to reduced metabolic activity under these conditions (Wilson and Griffin 1975).

The ten rhizobial strains in this experiment were significantly different in their growth response to drought. Two of the strains (UL 136 and UL 222) showed greatest growth and five of the strains (UL 61, UL 150, UL 167, UL 201, and UL 207) showed low growth at -1.0 and -1.5 MPa. The ability to tolerate drought varies with species and strain of rhizobia (Barnet et al. 1988). Bushby and Marshall (1977) evaluated

TABLE 4. Growth and survival of *Rhizobium meliloti* strains at soil moisture potential -1.5 MPa.

Strain	Time (Days)					
	0	4	8	14	21	28
	(Log number of rhizobia g ⁻¹ soil)					
UL 61	7.975 a	8.007 b	7.518 a	6.818 ab	6.728 a	6.664 a
UL 115	7.977 a	7.615 a	7.623 b	7.554 c	7.266 b	7.153 c
UL 129	8.184 b	7.667 a	7.606 b	7.569 c	7.193 b	7.065 b
UL 136	8.495 c	8.127 c	8.045 c	7.935 d	7.786 c	7.647 d
UL 150	8.138 b	7.945 b	7.512 a	6.874 b	6.731 a	6.680 a
UL 167	8.172 b	7.968 b	7.495 a	6.788 a	6.742 a	6.731 a
UL 201	8.172 b	8.001 b	7.512 a	6.854 ab	6.766 a	6.702 a
UL 207	8.148 b	7.958 b	7.518 a	6.799 ab	6.765 a	6.691 a
UL 210	8.131 b	7.683 a	7.638 b	7.518 c	7.209 b	7.105 bc
UL 222	8.518 c	8.225 d	8.148 d	7.968 d	7.953 d	7.720 d

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

changes in rhizobial populations in sterile soils that were inoculated and then rapidly dried. Their results showed a rapid rhizobial mortality with rapid drying and documented differences among rhizobial strains in susceptibility to desiccation. Bushby and Marshall (1977) proposed that these differences in susceptibility among rhizobia were related to the amount of water retained by the cells during drought. Mahler and Wollum (1981), Mohammad et al. (1991) and Mary et al. (1994) also demonstrated that strains within species varied markedly in their ability to tolerate drought.

The growth of rhizobial strains was high at the start of the experiment but declined through time. Two of the strains (UL 136 and UL 222) showed significantly highest survival of viable cells after 28 days. Five of the strains (UL 61, UL 150, UL 167, UL 201, and UL 207) showed a low survival of viable cells after 28 days. The results of this experiment agree with those of Osa-Afiana and Alexander (1979), who observed a marked reduction in the viability of *R. trifolii* during the first two weeks of their experiment when soil water was above -0.8 MPa, and a more gradual decrease of viable cells after that. Miller and Pepper (1988) and Streit et al. (1991) also reported major reductions in numbers of rhizobia between the first and second week of their experiment with more gradual declines after 14 days. Mahler and Wollum (1981) found a decrease in numbers of cells of *R. japonicum* between one hour and one week with increasing drought from -0.03 to -1.5 MPa. Boonkerd and Weaver (1982) also showed that rhizobial populations from cowpea and peanut decreased rapidly during 45 days incubation in soil when exposed to dry conditions.

In the present study two *R. meliloti* strains (UL 136 and UL 222) showed greatest growth under low water potential and produced viable cell counts of more than 10^7 rhizobia g^{-1} of soil. Regulations on inoculant quality for effective nodulation vary among countries and no international standards exist. The acceptance limit of inoculant in the U.S.A. and Canada is 9.4×10^6 rhizobia g^{-1} of soil (Olsen et al. 1994). The viable cell counts of strain UL 136 and UL 222 are within the range of the U.S. standard, and these strains probably could be used successfully as inoculants in arid and semiarid environments. As a result, there apparently are opportunities to select drought-tolerant rhizobial strains from Pakistan.

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WPLYW POTENCJAŁU WODY
NA WZROST I PRZEŻYWALNOŚĆ RHIZOBIIÓW LUCERNY W GLEBIE

STRESZCZENIE

Wzrost i przeżywalność rhizobiiów lucerny (*Rhizobium meliloti* Dang.) z Pakistanu i Nepalu badano w fiolkach wypełnionych sterylną glebą przy -0,03, -1,0 i -1,5 MPa. Główne efekty poziomu wody, szczepów rhizobialnych i długości ekspozycji na wysychanie oraz ich interakcje wykazały wysoce istotny ($P < 0,001$) wpływ na liczbę rhizobiiów glebowych. Wzrost i przeżywalność rhizobiiów były najwyższe w glebie przy -0,03 MPa a następnie w glebach o -1,0 i -1,5 MPa. Najwyższe liczby komórek obserwowano w szczepie UL 136, następnie w szczepie UL 222, najniższą w szczepie UL 61. Dwa szczepy rhizobialne (UL 136 i UL 222) tolerowały wysychanie najlepiej i wykazywały najwyższy wzrost i przeżywalność przy niskim potencjale wody. Te dwa szczepy można by prawdopodobnie wykorzystać jako inokulanty do uprawy w suchym i półsuchym środowisku.

SŁOWA KLUCZOWE: rhizobia lucerny, tolerancja na wysychanie, inokulanty, potencjał wody w glebie, gleba sterylna.