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Differential Tolerance in Clover Rhizobia to Moisture and Heat Stresses

A.A.Trotman and R.W. Weaver

Summary

Rhizobia on inoculated clover seed are often subjected to environmental stresses after sowing in the fall. Numbers of effective rhizobia present in the area of early root development will determine the efficacy of inoculation. In the laboratory, rhizobial isolations were made from nodules collected from Arrowleaf plants grown in soil from 15 locations in Texas. Ten commercial strains and 150 field isolates were added to sterilized soil and subjected to low moisture (-1.5MPa) and heat (37°C) stresses to determine their ability to survive. The commercial strains were not very tolerant of either moisture or heat stress. Of 10 commercial inoculant strains included in this experiment, a decrease in population size of at least a thousandfold occurred for seven strains under conditions of low moisture stress for a period of 7 days. For the other three strains, populations declined at least a hundredfold by 7 days. Under similar conditions of moisture stress, 17 of 150 field isolates maintained at least 10% of their original population. When under heat stress, three inoculant strains showed a thousandfold decrease in population size and only one showed less than a hundredfold decline by 7 days. Twenty field isolates showed enhanced survivability when under heat stress by maintaining at least 10% of their initial population. The moisture resistant field isolates were not as effective in nitrogen fixation as the commercial inoculant strain.

Introduction

Early establishment of the dinitrogen-fixing association between rhizobia and arrowleaf clover (*Trifolium vesiculosum* Savi.) is essential for good nodulation and seed ling establishment. Arrowleaf clover seeds are routinely inoculated with clover rhizobia (*Rhizobium leguminosarum* bv. *trifolii*), but little consideration is given to the influence of prevailing environmental conditions on rhizobial survival. In the fall when clover is sown the rhizobia are exposed to desiccation coupled with high temperature which present severe stress conditions (Weaver et al. 1985). If low moisture

TABLE 3. EFFECT OF MOISTURE-STRESS (-1.5 MPa) ON SURVIVAL OF 150 ISOLATES OF CLOVER RHIZOBIA IN SOIL AT VARIOUS TIMES AFTER INOCULATION

Tolerance Ranking*	Number of Isolates	Time after Inoculation: Hours 4	Days 1	Days 4	Days 7
		Percent of Added Cells Surviving			
1	66	12.60**	0.25	0.03	<0.01
2	37	3.20	7.97	0.15	<0.01
3	30	15.80	3.20	3.20	0.03
4	17	20.00	31.70	31.70	10.00

* The isolates were ranked from most sensitive (1) to least sensitive (4) to moisture stress.
** Numbers represent the average for the isolates in each pattern.

TABLE 4. EFFECT OF HEAT STRESS (37°C) ON SURVIVAL OF 150 NODULE ISOLATES OF CLOVER RHIZOBIA IN SOIL AT VARIOUS TIMES AFTER INOCULATION

Tolerance Ranking*	Number of Isolates	Time after Inoculation: Hours 4	Days 1	Days 4	Days 7
		Percent of Added Cells Surviving			
1	98	3.36**	0.12	<0.01	<0.01
2	21	12.60	26.90	15.90	<0.03
3	11	17.20	5.40	12.30	<0.01
4	20	3.16	19.90	20.00	10.00

* The strains were ranked from most sensitive (1) to least sensitive (4) to heat stress.
** Numbers represent the average for the isolates in each pattern.

The findings in this study indicate that there is variability in soil isolates with respect to survivability under stress. Those strains currently used in inoculants for arrowleaf clover are not as tolerant of low moisture or heat stresses as many of the field isolates. Unfortunately, the field isolates having the ability to tolerate the stress conditions exhibited poor dinitrogen-fixing effectiveness. For the best commercial inoculant strains 99% of the cells died by 7 days indicating a need for finding strains with the ability to tolerate low moisture and heat stresses. There were field isolates in this study which showed an ability to tolerate these stresses. The dinitrogen-fixing potential needs to be introduced into the stress-tolerant strains, perhaps through genetic engineering.

Literature Cited

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or heat delays germination of the arrowleaf seed (Evers 1980) then nodulation of the clover is reduced (Rich et al.1983). Placing the inoculated seed below the soil surface enhances the survival of rhizobia on seed (Weaver 1988). Although improved inoculation techniques can influence survival it would be advantageous to have rhizobia with an inherent ability to better survive heat and low moisture stress. We obtained 150 isolates of clover rhizobia and screened them for their tolerance to desiccation and heat.

Procedure

Soil samples collected from 15 locations, in Texas, all having been previously seeded to clover, were sieved (2mm) and sown to arrowleaf clover. After 6 weeks, plants were harvested and 10 nodule isolates were obtained from plants grown in soil from each location. Stress tolerance of 150 field isolates was studied using a completely randomized design with three replications and two treatments. Treatments involved moist soil (-0.03 MPa) incubated at 37°C to test heat tolerance of isolates and dry soil (-1.5 MPa) incubated at 28°C to test for tolerance of low moisture stress. Vials with sterilized soil were weighed before and after inoculation and after incubation for moisture determination. In the heat stress treatment, moisture was maintained at -0.03 MPa with the use of humidity chambers when incubating soil samples at 37°C. Sterilized soil (5 g) was inoculated with 3-day-old rhizobial culture. Inoculated soil samples were incubated and rhizobial population size determined at sampling times of 0, 1, 4, and 7 days after inoculation. Population size was determined by tenfold serial dilution and plate counts made of soil samples followed by plate counts on yeast mannitol agar (YM). Plates were incubated for 3 to 5 days at 28°C and colonies counted. The numbers are reported as percent of initial inoculum added as broth culture. The dinitrogen-fixing effectiveness of selected isolates was determined in a glasshouse pot study. A commercial inoculant strain and 17 field isolates were used to inoculate (Yuchi) arrowleaf clover seeds. A completely randomized design with four replications was utilized for each strain.

Results and Discussion

Rhizobia varied greatly in their response to moisture and heat stress. For 7 of the 10 commercial inoculant strains, the population decreased at least a thousandfold by 7 days under moisture stress. The population of the other three commercial strains decreased at least a hundredfold by 7 days (Table 1). When the response of the commercial strains to heat stress is examined, those strains which showed tolerance to heat stress (115-5 and 162X97) were not those which were tolerant of the low moisture stress treatment (Tables 1 and 2). Three of the inoculant strains (162X68, 162K13, and 114-6) showed a thousandfold decrease in population size under heat stress, (Table 3). Also, those commercial strains having the best survival under heat stress conditions unfortunately also showed a greater than 90 percent decline in population size by 7 days. This finding further demonstrates the need to take into consideration the effects of environmental conditions on inoculated seeds.

Under heat stress conditions there were 38 of 150 field isolates showing a thousandfold decrease in numbers by 7 days. As evident under moisture stress conditions, the field isolates showed differing profiles in response to heat stress, (Table 4). There were 66 field isolates which showed a pattern of continuous decline in population size over 7 days when incubated under heat stress. Some of the field isolates also had declining numbers initially followed by an increase or an initial increase followed by a decrease. Of the field isolates tested, 17 showed a trend for reduced susceptibility to heat stress. Of significance was the observed difference in tolerance of heat or low moisture in the field and inoculant rhizobia. Those rhizobia tolerant of low moisture stress were not always the ones to be tolerant of heat stress. However, there were four cases where the same field isolates were both tolerant of low moisture and heat stress.

TABLE 1. EFFECT OF MOISTURE STRESS (-1.5 MPa) ON SURVIVAL OF COMMERCIAL STRAINS OF CLOVER RHIZOBIA IN SOIL AT VARIOUS TIMES AFTER INOCULATION

	Time after Inoculation			
	Hours	Days		
Strain	4	1	4	7
	Percent of Added Cells Surviving			
162X95	8	0.02	<0.02	<0.02
TA1	18	0.11	<0.01	<0.01
115-5	23	0.14	<0.01	<0.01
114-2	13	0.08	0.01	<0.01
114-6	20	1.60	0.01	<0.01
162K13	1	0.17	0.14	0.01
162X97	16	3.98	2.00	<0.01
162Y14	20	3.20	0.01	0.02
162X68	8	3.98	0.20	0.10
162Y10	13	3.32	3.32	0.32

TABLE 2. EFFECT OF HEAT STRESS (37°C) ON SURVIVAL OF COMMERCIAL STRAINS OF CLOVER RHIZOBIA IN SOIL AT VARIOUS TIMES AFTER INOCULATION

	Time after Inoculation			
	Hours	Days		
Strain	4	1	4	7
	Percent of Cells			
TA1	36	28	<0.18	<0.18
162Y14	8	63	<0.01	<0.01
162K13	22	11	<0.01	<0.01
162X95	5	2	<0.02	<0.02
162X68	20	10	<0.01	<0.01
114-2	5	3	0.33	<0.06
162Y10	17	3	2.10	<0.03
114-6	6	8	0.10	<0.01
162X97	4	9	0.63	0.05
115-5	11	29	<0.01	0.36