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Spatial Variability in the Population of Chickpearhizobia in Relation to Soil Moisture and Other Soil Characters.

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ABSTRACT

Survey of eleven sites in northern that was carried out to determine the status of the rhizobium population in relation to soil moisture, soil particle size components and certain chemical characters of soil. Chickpea-rhizobium population varied significantly (F= 38.54, P=0.000) from site to site. Half life of rhizobia at test soil moisture levels (5%, 4%, 2%, 1%, 0.5%) decreased with decreasing moisture under laboratory conditions. Significant negative correlation (r>0.7) between rhizobium population and soil moisture was recorded. Non-significant correlation between in situ rhizobium population and soil moisture indicated that a protective mechanism is operative in the dryland ecology under study. Significant positive correlation (r=0.56, P<0.001) between soil phosphorus and chickpea-rhizobium poulation indicates that the survival mechanism may be pmediated in the absence of the host. Multivariate linear analysis indicated that phosphorus, total bacteria, clay content, Magnesium and the soil moisture were the main factors affecting rhizobium poulation in the area/ecology.

INTRODUCTION

Chickpea is the most important leguminous crop of Pakistan. It is grown over an area of 0.738 mha. This region lies between latitude 29°58'N 32°35' and longitude 71°43"E to 72°18'E receives mean annual precipitation of around 160-300mm (Khan et al, 1991). The soils are sandy chickpea is the sole crop grown during the year.

Reawakening of consciousness to the use of biofertilizer-s/microbial inculants for sustainable agriculture has provoked many investigations (Wani et al, 1995) on rhizobial inoculation and related ecological aspects. This technology bears great promise for semiarid and rainfed ecologies like Thal. The potential of this technology can best be exploited if knowledge on populations of indigenous rhizobia and its variability with soil moisture and other soil factors is obtained. This information can help in developing inoculation strategies in this type of region. Studies on the survival of rhizobia with reference to temperature and moisture stress have been recommended (Rupela and Beck, 1990; Brockwell *et al*, 1995; Vincent, 1988; Venkateswarlu, 1992).
The present study is also significant from the viewpoint of the role that the poulation size of indigenous rhizobia play in the establishment and symbiotic performance of inculant rhizobia, especially under dryland conditions.

Materials and Methods :

Soil sampling: Chickpea field at 11 sites of northen thal were collected with the help of a PVC pipe peice (15cm x 3.5cm) core being supported by a lid underneath, just after germination, from between the rows in quadruplicate from each field, in airtight tins for moisture determination and in polythene bags for population estimation and for physical and chemical analysis.

Enumeration of chickpea-rhizobia : Ten grams of each composite soil sample was suspended in 90ml. of sterilized distilled water and allowed to shake on a rotary shaker for 30mins. Four fold serial dilutions were prepared. Four fold serial dilutions wre prepared. Soil dilutions from 4^{-1} to 4^{-6} with four replicate units per dilution were used to determine number of rhizobia/g of soil by MPN plant infectivity method (Speidel and Wollum, 1980).

Enumeration of total bacteria : Total bacterial population was estimated by plating 0.1 ml of tenfold serial dilutions on Yeast Mannitol Agar (Somasegaran and Hoben, 1985) medium containing congo red. Number of bacteria/g soil was calculated from the colony forming units obtained on plates.

Soil moisture determination: Soil moisture content was determined gravimetrically by drying soil at 100^{o}c in a dry air oven.

Other soil characters: Soil particle size component was determined following love *et al* (1977). All other soil characters were analyzed by following Winkleman et al (1986).

Survival of chickpea-rhizobia at different moisture levels: Three rhizobial isolates (Sk-8, BN8, S2a, having ability to grow equally well at 40^{o}C as at 17^{o}C.) were subjected to five different soil moisture levels (0.5%, 1%, 2%, 4%, 5%) in polypropylene bags, injecting an initial population of 10^{7}-10^{8}/g of rhizobia into sterilized Thal soil. The bags were incubated at 40^{o}c for a period of 72 days. The population was estimated by dilution plate count method on Yeast Mannitol Agar medium (Somasegaran and Hoben, 1985), at appropriate time intervals. The response of rhizobia to water stress was determined in terms of 50%-survival (half life) by plotting regression line between number of rhizobia and time-period, for each set of strain and moisture level according to Yarwood and Sylvester

(1959). Data was analyzed using Mstatc software, wherever necessary. Simple correlation between individual characters and chickpea-rhizobium population as well as multivariate linear regression was worked out to find the significance of various soil characters.

Results and Discussion :

The area surveyed represents chickpea-fallow cropping system. The soil moisture varied from 1.066% to 5.88% from site to site (Table 1) at a depth of 6-15 cm. just after germination. Soil moisture differed significantly (F=26.15, P=0.000) from site to site. MPN of total soil bacteria varied between 10^4/g and 10^8/g soil at different sites (Table 1). MPN of chickpea-rhizobia varied from 10^2/g-10^5/g. Rhizobium population differed significantly (F=38.54, P=0.000) from site to site. Previously chickpea-rhizobium population of 10-103/g of soil has been reported in sandy soils of Rajastan (Rupela *et al*, 1987) having chickpea-maize cropping system. All the soil physical and chemical characters viz. clay (Table 2), pH, organic matter, NO_3, Phosphorus, Potassium, Calcium, Magnesium and Sodium, showed significant variability (Table 3) from site to site.

Out of 11 factors, rhizobium population showed significant simple correlation with phosphorus (r=0.584, P<0.001) and total related bacterial population (r=0.59, P<0.001). Chickpea-rhizobium population as well as total bacterial population showed non-significant correlation with the soil moisture. In order to obtain a more realistic estimate of the contribution of factors, multiple regression analysis was worked out. Soil characters were found significant in the following order Phosphorus> total bacterial> clay> magnesium> soil moisture.

Step-wise regression analysis showed that Total R(=0.85) was significant (F=25.6). The following equation was formed

Number of rhizobia = -4.31 + 0.00000034P + 0.000016Mg + 0.00000024tb + 0.00076clay + 0.017

It indicates that out of 11 factors only four played highly significant role (table 4) in deteremining chickpea-rhizobium population in the following order. P>Mg>total bacteria>clay-content. These reults indicate that rhizobia i) either possess intrinsic ability to survive soil moisture levels of 1% or even lower during hot summer or ii) some protective mechanism is operative through phosphorus and clay. Total bacteria also seem to play significant role. The later hypothesis is supported by the results of the laboratory experiment with selected indigenous rhizobia (SK-8, BN8, S2a). Under five soil moisture levels viz.0.5%, 1%, 2%, 4%, 5% of loamy sand, collected from Thal, significant correlation (r >0.78, p=0.05) with soil moisture level was recorded in case of each rhizobium (Fig.1), at 40^{o}c, which represents the summer temperature of sand at a depth of 6-15 cm, in the region. Over the period of 72 days incubation at 0.5% soil moisture, population of chickpea rhizobia declined from 10^{7} to 10^{2}-10^{0}/g. Though all the test rhizobia showed their sensitivity to moisture stress, highest S50-period (45 days) at 0.5% moisture level was recorded in case of SK-8 (Table 2). The same strain showed lowest slope value (b=-0.063) at 0.5% soil moisture. Closely related microorganism, *Pseudomonas spp.* has been reported to survive for less than one week in sandy soil having 5%-10% moisture (Dealto & Surico, 1982), at 27^{o}C.

Controversial reports have been made regarding the correlation of survival of rhizobia and their population density with soil factors (Osa-afiana and Alexander, 1982; Chao and Alexander, Boonkerd and Weaver, 1981) in cowpea. The present study was the first attempt to correlate soil characters to natural population of chickpea-rhizobium at different sites in same ecology. The possibility of protective factors among soil characters was sought. Soil pH varied between 7.3-8.27 from site to site (Table 3). No simple correlation could be found. Multiple regression analysis also indicated it as non-significant factor. Similar observations are reported by Rupela *et al* (1987) for a pH range 6.7-7.8 at farmers' field in case of the populations of *R. phaseoli* and *melioti*.

Soil organic matter varied from 0.19% to 0.62% from site to site (Table 3). It showed non-significant correlation (r=0.16, p=0.3) with MPN count of chickpea-rhizobia. Multiple regression analysis also indicated it as non-significant factor. Though organic matter in concentration less than 2% has been reported to proportionately influence the population and hence survival of rhizobia, yet no report exists on any effect of such low range as recorded here.

Nitrate -N of soil varied from 0.07 to 1.82 ug/g from site to site (Table 3) and MPN of total bacteria and chickpea rhizobia showed non-significant simple correlation. Multi regression analysis indicated it as a non-significant factor. Soil nitrate is rarley a limiting factor for rhizobium population, as a very low concentration is required.

Phosphorus (extractable) varied from 4ug to 9ug/g of soil (Table 3) from site to site. Total bacterial population showed non-significant correlation, while chickpea-rhizobium population was found significantly correlated (r=0.56, p<0.001) with phosphorus-content. Multiple regression analysis also indicated it as highly significant factor in determining rhizobium population. Soil phosphorus is thought to promote the production of extracellular polysaccharides (EPS) in rhizobia (Bushby, 1982). Hence soil phosphorus may be helping in the survival of rhizobia through the production of EPS under dry conditions. Difference in response to moisture stress by rhizobia under sterilized and unsterilized soil suggest that phosphorus may be playing role as a product of another living organizm, probably phosphorus-solubilizing bacteria.

Potassium varied from 53ug-61ug/g Clcium from 0.28% to 0.62%, magnesium from 40ug to 140ug/g while sodium varied from 100ug to 600ug/g of soil . Rhizobium population showed non-significant correlation with either of these elements. However, multiple regression analysis indicated Mg as highly significant factor. Viability of rhizobia/1 has been reported to be affected by amount of Ca below 80ug/1 and of Mg below 260ug (Trinick, 1982).*R.trifolii* has been reported to respond to a Potassium concentration of upto 234ug/1 (Trinick, 1983). The present situation depicts a K-deficient as well as Magnesium-dificient situation. An improvement in Mg-status of soil may help in better survival of rhizobia. Need of collecting evidence for the role of clay as protective agent for rhizobia has been suggested by Venkatesvarlu (1992). Soil particle size component analysis indicates that at all the sites except one, the soil is loamy sand. Clay-content (<0.2u) varid from 8.0% to 12.67% from site to site.MPN of chickpea-rhizobia showed no significant correlation with clay-content. However MPB of total bacteria showed significant negative correlation ($r= -0.61, p<0.001$) with this soil character. This negative relationship can not be considered as a procedural deficiency because soils having such clay contents have been reported to release adsorbed baceria on mere physical shaking (Rupela *etal*, Bezdicek and Danaldson, 1980). Also the clays in the region are uncharged type of clays (unpublished data of the author), which have low capacity to establish bacterial adhesion onto them. Multiple regression analysis indicated that clay played second significant role to Phosphorus in determining rhisobium population in the ecology (Table 4). Soil clay content has been reported to afford protection under dry soil conditions for rhizobia (Osa-afiana and Alexander, 1970; Heijnen and Veen, 1991). The present results support this concept. Survival of mesquite-rhizobia in sandy clay loam of Sonoran desert, having 26% clay content and 0.3% organic matter was reported to be better over a period of 28days at field capacity (Shoustari and Pepper, 1985).

Since Kaolinite, mica and illite are the dominant clays in the region (Abdullah, 1987, unpublished data of the author). These uncharged minerals have been reported not to play any role in the protection of rhizobia under dry conditions (Chao & Alexander, 1982). On the other hand improvement in the survival of a fast grower *R.leguminosarm* has been reported by the addition of kaolinite to loamy sand (Heijnen and Veen, 1991). The present results urge to find out the basis of protection of rhizobia and other related bacteria by uncharged clay.

It can hence be concluded that chickpea-rhizobia were sensitive to water deficit stress in the range of 0.5% to 5% under laboratory conditions. Soil moisture though played significant role in determining the chickpea-rhizobia population in natural environment. It's effect can however be controlled by monitoring the amount of Phosphorus, total bacteria, and clay in such loamy sand of arid regions.

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Table 1: Population of indigenous rhizobia and related bacteria /g soil at different soil moistures.

Name of site	Soil Moisture (%by weight)	MPN of chickpea rhizobia (log_{10})	MPN of related bacteria (log_{10})
Kalurkot	1.06±0.46 d	2.70± 0.09 fg	4.06± 0.16 f
Zamaywala	1.35±0.03 cd	5.15± 0.23ab	5.66± 0.27 d
Rodi	1.72± 0.15 cd	2.40± 0.08 g	4.76± 0.33 e
Fazal	1.06± 0.19 d	4.45± 0.13 c	6.71± 0.72 bc
Dullewala	2.24± 0.21 c	5.30± 0.36a	6.82± 0.25 bc
Rakhmahuta	3.74± 0.41 b	3.15± 0.26 ef	6.56± 0.40 bc
Khanser I	4.17± 1.62 b	3.46± 0.43 de	4.78± 0.30 e
Bhattianwala	5.25± 1.11a	3.81± 0.22 d	7.14± 0.64 ef
Dagarkotli	1.53± 0.32 cd	5.11± 0.48ab	4.25± 0.55 c
Khanser II	5.88± 0.76a	4.50± 0.62 c	6.47± 0.37 b
Rakhghulaman	1.61± 0.46 cd	4.81± 0.29 bc	8.54± 0.44a
$LSD_{0.05}$	1.01	0.46	0.62

Table 2 : Half life (S50) in days of different rhizobia different soil moisture levels.

Strain	Soil Moisture level				
	5%	4%	2%	1%	0.5%
SK-8	120	100	66.3	52	45
BN8	110	100	53.6	48.3	41.7
S2a	75	65.5	36	36	32.5

Table 3: Soil Phsico-chemical characters at different sites.

Site	pH	Organic matter(%)	Clay(%)	NO3-N(%)	P (ppm)	K (ppm)	Ca(%)	Mg(ppm)	Na(ppm)
S2	7.5±0.08c	0.33±0.04d	12.0±0.16c	0.07±0.02fg	3.95±0.06g	55±2.2def	0.37±0.02d	40±3.74d	460±2.16
S3	7.6±0.08c	0.52±0.01b	11.2±0.08d	0.39±0.02d	9.33±0.29a	49±2.9gh	0.35±0.02d	60±2.38c	400±2.83c
S4	8.0±0.18b	0.50±0.03b	11.0±0.10e	0.21±0.03ef	4.14±0.13fg	56±2.4cde	0.52±0.04	100±1.83b	220±3.16d
S5	8.3±0.14a	0.10±0.02f	10.0±0.08h	0.37±0.03d	9.07±0.19a	60±1.29ab	0.28±0.04e	40±3.56d	140±3.46f
S6	7.9±0.01b	0.62±0.02a	10.6±0.08g	1.06±0.03b	5.09±0.37c	61±1.15a	0.46±0.03c	140±4.04a	600±3.56a
S7	8.3±0.08a	0.52±0.04b	8.58±0.02j	0.39±0.02d	5.38±0.52c	54±4.27ef	0.37±0.02d	60±2.94c	120±3.16g
S8	8.0±0.02b	0.40±0.06c	12.6±0.01a	0.0±0.01g	4.54±0.14e	48±2.83h	0.23±0.02f	60±1.30c	140±4.83f
S9	8.2±0.08a	0.19±0.02e	9.53±0.01i	0.56±0.05c	4.33±0.19ef	53±2.75ef	0.62±0.06a	140±3.59a	120±2.16g
S10	8.0±0.00b	0.35±0.05d	12.18±0.01b	0.25±0.03de	6.48±0.16b	57±1.83bcd	0.38±0.03d	60±2.58c	100±4.4h
S11	8.2±0.00a	0.64±0.05a	12.68±0.01a	2.82±0.06a	6.22±0.06b	52±1.71fg	0.37±0.02d	60±3.16c	160±4.2e
S12	7.3±0.08d	0.48±0.03b	10.7±0.01f	0.21±0.03ef	4.88±0.10d	60±3.16bc	0.52±0.03b	40±5.69d	120±3.92g
$LSD_{0.05}$	0.13	0.046	0.102	0.14	0.34	3.68	0.05	4.85	5.10

Table 4: Multiple Regression Analysis.

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-4.307730592	1.130228071	-3.811381	4.79164E	-6.593830402	-2.021630782
P	0.322651909	0.052621814	6.131523	3.40054E	0.216214349	0.42908947
Mg	0.013648546	0.002772288	4.923206	1.59539E	0.00804107	0.019256021
Tm	0.424301775	0.06792703	6.246434	2.35516E	0.286906523	0.561697027
Clay	0.271076067	0.074223564	3.652156	7.62675E	0.120944886	0.421207247