

EFFICACY OF RHIZOBIUM STRAINS FOR GROUNDNUT INOCULATION UNDER RAIN FED CONDITIONS

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The efficacy of symbiotic association between three *Rhizobium* strains i.e. B (TAL-1371, C (Tal-1000) and D (NC-92) and two groundnut genotypes viz. V₁ (ICG-4993) and V₂ (ICG-732) was studied in a field trial under rain-fed conditions of Pothowar in a semi-arid environment. Inoculation increased symbiotic and yield traits of groundnut genotypes. Plants inoculated with strain D (NC-92) had significantly more nodules plant⁻¹, nodule dry weight plant⁻¹, N content of nodules, roots and shoots of both V₁ (ICG-4993) and V₂ (ICG-7326) than that of strain B (TAL-1371) or strain C (TAL-1000). The strain D (NC-92) exhibited specificity and positively interacted in influencing symbiotic traits of V₂ (ICG-7326). Plants inoculated with strain D (NC-92) produced more pods plant⁻¹, pods and biological yield ha⁻¹ compared to strain B (TAL-1371) or strain C (TAL-1000). The effect of strain B (TAL-1371) and strain C (TAL-1000) was statistically same on symbiotic and yield traits of groundnut genotypes. *Rhizobium* strain D (NC-92) was found superior to strain B (TAL-1371) or strain C (TAL-1000) in their effects on symbiotic traits and groundnut yield.

Key words: *Rhizobium leguminosarm*, strains, groundnut genotypes, symbiotic and Yield traits.

INTRODUCTION

Groundnut is commonly grown on coarse-textured soils in rainfed areas of Pothowar. In general, fertility status of soils in barani tract is low and indeed, these soils have very low organic matter content i.e. 0.2–1.2% (Ahmed *et al.*, 1988). Nitrogen estimated from organic fractions of rain fed soils of Pakistan ranges from 0.03 - 0.07% (Frederich *et al.*, 1991). Low soil nitrogen is a major factor for inefficient water use and low crop yields in rainfed areas of Pakistan (Khan *et al.*, 1989). A significant increase in legumes yield has been attributed to *Rhizobium* bio-fertilizer used at research station in field trails (Sattar *et al.*, 1996) but inoculation effects were not consistent at farmer's fields due to the competition of indigenous *Rhizobium* strains with inoculum strains (Sexena *et al.*, 1996) or unfavorable climates (Ashraf *et al.*, 2002). The symbiotic partnerships between *Rhizobium* and legumes vary widely in their degree of specificity (Lesely and Young, 2004) and researchers have reported the specificity of *Rhizobium* strains by soybean genotypes (Ashraf *et al.*, 2002), *Rhizobium* strains by pea genotypes (Santalla *et al.*, 2001), *Rhizobium* strains by mungbean genotypes (Rehman *et al.*, 2002). Competitive ability of an inoculant strain is a key requirement for successful colonization of plant roots, nodule formation and N₂-fixation (Sessitch *et al.*, 2002). Herrige and Rose (2000) have also pointed out the importance of cultivar- strain interaction for improved N₂-fixation in grain legumes production. Literature reviewed suggested that *Rhizobium* strains be selected for their tolerance to extreme soil conditions or unfavorable climate or strain x genotype specificity or

competitiveness with indigenous *Rhizobia* strains to prepare inocula for improving legume productivity in rainfed areas. The present study was planned to evaluate efficacy of symbiotic association between *Rhizobium* strains and groundnut genotypes under rainfed conditions. The information about quantitative differences in symbiotic traits and yield responses may be useful for plant breeding program and inoculation laboratories for improving N₂-fixation.

MATERIALS AND METHODS

A field experiment was carried out at research area, University of Arid Agriculture, Rawalpindi during kharif, 2001 to evaluate efficacy of association between *Rhizobium* strains and groundnut cultivars. The soil was clay loam and had an organic matter content 0.75%, available P 4.9 ppm, total nitrogen 0.4 mg kg⁻¹, saturation 34% and pH 7.8. A two factor factorial experiment was laid out in randomized complete block design with four replications having net plot size of 4 x 6 m. The following groundnut genotypes were tested: V₁ = ICG-4993 and V₂ = ICG-7326. Three strains of *Rhizobium* and an un-inoculated control were as: A = Control, B = TAL-1371, C = TAL-1000, D = NC-92. The strains of *Rhizobium* viz. TAL-1371, TAL-1000 and NC-92 for groundnut inoculation were obtained from Microbiological Laboratory, National Agricultural Center, Islamabad. The seeds were treated under shade with inocula of respective strains @ 10 g kg⁻¹ of seed just before sowing. The inoculants and half teaspoon of sugar were dissolved in water. The solution was sprayed on seeds and mixed thoroughly for uniform coating of inoculants on seeds. All plots of

each treatment combination were planted at seed rate of 65 kg ha⁻¹ in rows 40 cm apart with a single row hand drill on May 6, 2001. A week after emergence, thinning was done to maintain optimum plant population keeping 10 cm distance between plants.

A basal doze of PK @ 80:20 kg ha⁻¹ was blended in the soil at the time of seedbed preparation. All other agronomic practices were kept normal and uniform. Weeding was done thrice manually at 20, 40 and 90 days after sowing. Crop was harvested on October 28, 2001.

Five randomly selected plants for each treatment combination were dug out at flowering stage and washed carefully to count nodules plant⁻¹. The nodules from these plants were carefully removed, sun-dried and weighed to record data on nodule dry weight plant⁻¹. The same plants were separated into roots and shoot. The samples were ground and sieved to determine total N content of nodules, roots and shoots using the method proposed by Jackson (1982). Five randomly

The data were subjected to analysis of variance technique. F-statistic for main effect and interaction was based on residual mean square error. The LSD at 5% level of probability was used for comparison of treatment means (Montgomery, 2001).

RESULTS AND DISCUSSION

Symbiotic Traits

Main effect of inoculum stains and interactive effects between genotypes and inoculum varied significantly (Table 1). Plants of V₁ in combination with strain B, C and D treatments had 273, 379 and 518 more nodules plant⁻¹ than V₁ x A treatment. (control). Plants of V₂ in association with B, C and D treatments had 270, 350 and 496 more nodules plant⁻¹ relative to V₂ x A treatment (control). Effect of strain B and C for both V₁ and V₂ on nodules plant⁻¹ was statistically at par with each other but strain D increased nodulation by 22.63 and 11.66% over strain B and C, respectively in V₁ and

Table 1. Influence of inoculum strains on nodules number, nodule dry weight, and nitrogen content of nodules, roots and shoots of groundnut genotypes.

Treatment combinations		Nodules plant ⁻¹	Nodule Dry Weight plant ⁻¹ (g)	Nitrogen Content (%)		
Inoculums	Genotypes			Nodules	Roots	Shoot
A (Uninoculated)	V ₁	806.13	0.77	2.71	2.91	1.77
	V ₂	822.23	0.84	2.96	2.60	2.08
B	V ₁	1079.45	1.02	3.77	3.14	2.55
	V ₂	1091.90	1.20	3.38	3.27	2.21
C	V ₁	1185.55	1.08	3.51	3.02	2.66
	V ₂	1172.62	1.15	3.58	3.23	2.29
D	V ₁	1323.82	1.15	3.90	3.37	2.83
	V ₂	1317.97	1.30	4.09	3.46	2.90
Inoculum Means						
A (Uninoculated)		814.18	0.80	2.83	2.75	1.93
B		1085.67	1.11	3.57	3.20	2.38
C		1179.09	1.12	3.55	3.12	2.47
D		1320.90	1.22	4.00	3.41	2.87
Genotype Means						
V ₁		1098.74	1.01	3.47	3.11	2.45
V ₂		1101.18	1.12	3.50	3.14	2.37
LDS (0.05)						
Inoculum		97.69	0.10	0.26	0.36	0.28
Genotypes		-	0.07	-	-	-
Inoculum x Genotypes		138.20	0.15	0.36	0.51	0.39

NS = Non-Significant. LSD (0.05) =Least Significant Difference at 5% probability level. A: Un-inoculated Control, B: Strain Tal-1371, C: Strain Tal-1000, D: Strain NC-92, V₁: ICG-4993, V₂: ICG-7326.

selected plants were tagged to record data on pods plant⁻¹ at maturity. The pod and biological yield was recorded on plot basis and was converted into kg ha⁻¹.

by 20.7 and 12.39% over strain B and C, respectively in V₂, respectively. The strain B, C and D caused an increase of 32.46, 40.25 and 49.35% in nodule dry

weight plant⁻¹, respectively over control in V₁ and 42.85, 36.9 and 54.76%, respectively over control in V₂ (Table 1). Nodules N content of V₁ in combination with B, C and D was 39.11, 29.52 and 43.91% higher than V₁ x A (control), respectively (Table 1). Nodules N content of V₂ in combination with B, C and D was 14.8, 20.94 and 38.17% higher than V₂ x A (control). Roots N content of V₁ in association with B, C and D was 7.9, 3.78 and 15.8% higher than V₁ x A (control), respectively (Table 1). Root N content of V₂ in association with B, C and D was 25.76, 24.23 and 33.07% higher than V₂ x A (control). Shoot N content of V₁ in combination with B, C and D was 44.06, 50.2 and 59.88% higher than V₁ x A (control), respectively (Table 1). Shoot N content of V₂ in combination with B, C and D was 6.25, 10.09 and 39.42% higher than V₂ x A (control). Plants inoculated with strain D had significantly more nodules plant⁻¹, nodule dry weight plant⁻¹, N content of nodules, roots and shoots of both V₁ and V₂ than that of strain B or strain C. Analysis of symbiotic traits showed that strain D had established specifically more efficient symbiotic association with V₂ relative to other strains. The reason seemed to be more nodules dry weight of V₂ than V₁ in spite of that

having statistically same nodules number plant⁻¹ (Table 1). The results of this study conformed to the findings of Kumar *et al.* (1997) who screened compatibility of rhizobium strains in establishing symbiotic relationship in pot culture.

Pods and Biological Yield

Inoculum strains and interaction had significantly different effect on pods plant⁻¹ (Table 2). Plants of V₁ in association with strain B or strain C produced 27.55% more pods plant⁻¹ compared to control. Plants of this variety in association with strain D produced 35.63% more pods plant⁻¹ compared to control. Plants of V₂ in association with strain B, C and D produced 27.57, 22.70 and 49.43%, respectively more pods plant⁻¹ relative to V₂ x A (control). Inoculation improved 100-seed weight and plants inoculated with strain D produced 14.17% heavier seeds than that in control. Effect of genotypes and inoculum strain on pods yield ha⁻¹ was highly significant but interaction was not significant (Table 2). The V₂ significantly produced more pod yield ha⁻¹ than pod yield ha⁻¹ of V₁. Effect of strain B and C on pods yield ha⁻¹ was at par with each other and produced 16.32% more pod yield ha⁻¹

Table 2. Influence of inoculum strains on pods plant⁻¹, 100-seed weight, pods and biological yield of groundnut genotypes.

Treatment combinations		Pods plant ⁻¹	100-Seed weight (g)	Pods	Biological
Inoculum	Genotypes			(kg ha ⁻¹)	
A (Un-inoculated)	V ₁	51.35	40.53	1550.00	4033.33
	V ₂	53.30	41.15	1733.33	4250.00
B	V ₁	65.50	45.08	1783.33	4416.66
	V ₂	68.00	45.23	2016.66	4433.33
C	V ₁	65.25	44.70	1733.33	4516.66
	V ₂	65.40	46.23	2116.66	4450.00
D	V ₁	69.65	45.60	2033.33	4750.00
	V ₂	79.65	47.65	2366.66	4950.00
Inoculum Means					
A		52.33	40.84	1633.33	4133.33
B		66.75	45.25	1900.00	4416.66
C		65.32	45.46	1916.66	4483.33
D		74.65	46.63	2200.00	4850.00
Genotypes Means					
V ₁		62.94	43.97	1766.66	4433.33
V ₂		66.58	45.05	2050.00	4516.66
LSD (0.05)					
Inoculum		6.26	2.77	200	333.33
Genotypes		-	-	133.33	-
Inoculum x Genotypes		7.85	-	-	-

NS = Non-Significant. LSD (0.05) = Least Significant Difference at 5% probability level. A: Un-inoculated Control, B: Strain Tal-1371, C: Strain Tal-1000, D: Strain NC-92, V₁: ICG-4993, V₂: ICG-7326.

compared to control while plants inoculated with strain D produced 34.69% more pod yield ha^{-1} relative to control. Biological yield inoculated with strain B and strain C was not statistically different than control, however, biological yield ha^{-1} inoculated with strain D was 17.33% higher than un-inoculated control (Table 2). Plants inoculated with strain D produced more pods plant^{-1} , pods, and biological yield ha^{-1} compared to strain B or strain C. The effect of strain B and strain C was statistically same on symbiotic and yield traits of groundnut genotypes. *Rhizobium* strain D was found superior to strain B or strain C in their effects on symbiotic traits and groundnut yield. The results of this experiment are supported by the findings of Chetti *et al.* (1995) who reported that inoculation had significantly positive effect on 100-seed weight, pods yield and total dry matter production.

In summary, comparison of symbiotic and yield traits suggested that strains D was superior to strain B or strain C in establishment of symbiotic association with groundnut genotypes. Based on the results of these narrowly specified symbiotic interactions, it is concluded that these interactions are important component for improving N_2 -fixation in a sustainable agricultural production system.

REFERENCES

- Ahmad, N., J.G. Davide and M.T. Saleem. 1988. Fertility status of soils in dry land areas of Pakistan. *In: Proceedings of international seminar on dry land agriculture in Pakistan*. November 6–8, 1988. Fauji Fertilizer Co. Ltd. Lahore. pp. 22–49.
- Ashraf, M., F. Hassan and I.A. Chaudhry. 2002a. Interaction of rhizobium strains and lentil genotypes under rainfed conditions. *Pakistan J. Agri. Agril. Engg., Vet. Sc.* 18: 38–43.
- Ashraf, M., S. Ali and I. Hassan. 2002b. Interaction of rhizobium japonicum strains and soybean genotypes. *Pakistan J. Soil Sci.* 21: 49–54.
- Chetti, M.B., E. Entony, U.V. Mummigati and M.B. Dodamani. 1995. Role of nitrogen and rhizobium on nitrogen utilization efficiency and productivity potential in groundnut genotypes. *Farming Systems*. 11:25–33.
- Frederich, W.S., S.B. Wall, A. Rehman and M.S. Nawaz. 1991. Groundnut production in Pakistan. BARD Project, PARC, Islamabad. p. 81.
- Herridge, D. and I. Rose. 2000. Breeding for enhanced nitrogen in crop legumes. *Field Crops Res.* 65: 29–248.
- Jackson, M.L. 1982. Soil chemical analysis. Constable and Co. Ltd. London. 46.p.
- Khan, A.R., A. Qayyum and G.A. Chaudhry. 1989. A country paper on soil, water and crop management systems for dry land agriculture in Pakistan. *In: C.E. Whitman, J.F. Parr, R.I. Papendick and R.E. Meyer. (Eds). Soil, water and crop/livestock management systems for rainfed agriculture in near east region. ICARDA, USAID.* pp. 88–102.
- Kumar, T.K.D., A.R.M. Rao, S.M. Reddy, H.P. Srivastava, D.K. Purohit and S.R. Reddy. 1997. Screening of rhizobium isolates for symbiotic efficiency in pot cultures. *Microbial Biotechnology*. K.S. Bilgrami. Commemoration Vol. p. 75–78.
- Lesely, A.M. and J.P.W. Young. 2004. Diversity and specificity of *Rhizobium leguminosarum* biovar *viciae* on wild and cultivated legumes. *Molecular Ecol.* 13:2435–2444.
- Montgomery, D.C. 2001. Design and Analysis of Experiments. 5th Ed. John Wiley and Sons, New York.
- Rehman, M.A., N. Islam, A. Islam, M.K. Hassan and M.M.R. Talukder. 2002. Yield performance of mungbean (*Vigna Radiata* L). cv. Barimung-4 as influenced by Rhizobium inoculation. *Pak. J. Bio. Sci.* 5: 146–148.
- Sanatalla, M., J.M. Amurrio and A.M. deRon. 2001. Symbiotic interactions between Rhizobium leguminosarum strains and elite cultivars of Pisum sativum. *J. Agron. & Crop Sci.* 187: 59–68.
- Sattar, M.A., A.K. Podder and M.C. Chanda. 1996. Rhizobium Biofertilizer. *Proceedings of International Symposium on BNF associated with rice*. p. 15–20.
- Sessitch, A., J.G. Howieson, X. Perret, H. Antoun and E. Martinez-Romero. 2002. Advances in *Rhizobium* research. *Critical Review in Plant Sciences.* 21: 323–378.
- Sexena, A.K., S. Mittal, A. Dwivedi, K.V. Shashirala and B.R. Tilak. 1996. Competitiveness and symbiotic efficiency of potential inoculant's strains of *Bradyrhizobia* Species in *Vigna*. *Microbiol. Res.* 15: 219–224.