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EFFECTIVENESS AND COMPETITIVE ABILITY OF THE ANTIBIOTIC RESISTATNT MUTANTS OF *RHIZOBIUM* SP.*

Infectiveness and effectiveness are two genetic characters of the genus *Rhizobium* which permit the organism to enter into a symbiotic association with the host legume to form nodules for nitrogen fixation. A method used in genetic studies on *Rhizobium* for isolation of ineffective and non-ineffective mutants is the selection of strains resistant to certain antibiotics. However, the results reported so far present a controversial picture of the relationship between resistance and symbiotic nitrogen fixation of *Rhizobium* (Damary and Alexander, 1969, Zelazna—Kowalska, 1971, Levin and Montgomery, 1974).

Present study was carried out to determine the effectiveness and competitive ability of the mutants of *Rhizobium* sp. to streptomycin, erythromycin and kanamycin, Native *Rhizobium* sp. from nodulating pigeonpea *(Cajanus cajan* Mill sp.) plants was isolated and a c!onal population was raised which was designated as AS_1. Yeast extract mannitol (YM) was used as the growth medium. Exponentially growing cells of AS_1 were plated on YM plates containing 10,100 and 500 μg/ml of the each antibiotic (streptomycin, erythromycin add kanamycin) and spontaneous antibiotic resistant mutants were isolated randomly (Vincent, 1970).

Plant nodulation test was carried out in pigeonpea cultivar Bahar in a randomised block design with nine replications of each treatment. Surface sterilized seeds of pigeonpea were germinated under aseptic conditions. The 48h old seedlings were transferred to the agar slants prepared in large glass tubes (200 x 38 mm) supplemented with Thornton's nutrient medium (Vincent, 1970) and 1 ml of exponentially growing cultures of *Rhizobium* strains under test was poured on the roots- When the seedlings attained appropriate height, the shoot portion was pushed out gently under aseptic condition. Plants were grown under fluorescent lights in a growth chamber on an 18 h/day illumination at 27 + 2°C and were watered regularly with Thorton's broth. After sixty days, the plants were taken out and length of the shoot, total number of noduies per plant, dry weight of the nodules, plant dry weight as well as total nirogen content per plant (Micro-kjeldahl method) were determined.

Based upon the data regarding shoot length, number of nodules per plant, nodule dry weight, plant dry weight and total nitrogen content per plant of the inoculated pigeonpea plants (Table 1), all the antibiotic resistant mutants were classified into four groups viz., (i) Non-infective (kan_3) (ii) Partially effective (str_1, str_2, str,, ery_1, ety, and kan_2) (iii) Effective (kan_1) and (iv) Very effective (ery_2).

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Strain kan_3 was non-infective whereas ery_2 showed hyper efficiency in nitrogen fixation. This hyper efficiency might be due to increased activity of nitrogen fixing (*nif*) genes (Jagat Ram, 1979).

The loss of infectiveness of streptomycin resistant mutants (Maier and Brill 1978J and kanamycin resistant mutants(Damery and Alexander, 1969) was due to an altered cell wall and lipopoiysaccharide suggesting that the lipopoiysaccharide was a receptor for plant attachment. Mutants with altered cell wall and lipopolysaccharide may not form nodules (Vincent, 1980). Here also the non-infectivity of kan_3 might be due to the above explained reason.

Out of the nine mutants isolated, only two mutants namely ery_2 and kan_1 were effective in nitrogen fixation. This result indicates that these mutants may not have defect in general cell surface or function. However, in this study the rest of the mutants (str_1, str_2, str_3, ery_1, ery_3 and kan_2) were inferior to the parent strain This varied effect of nitrogen fixation might be due to different mechanisms of resistance in such cases.

These marked effective rhizobial strain can be used for the competition studies for knowing the efficiency of field inoculation.

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Table 1

Different plant characters of pigeonpea plants inoculated with parent and mutant strains of *Rhizobium* sp.

Treatment	Length of shoot (cm)	Total no. of nodules	Nodule dry weight (mg)	Dry weight of plant (mg)	Total N/ plant (mg)
Control	25.83	*	*	159.00	5.82
AS_1	30.33	20.33	12.66	191.00	9.33
str_1	29.66	19.66	9.33	180.00	6.83
str_2	28.83	17.66	12.00	184.66	8.85
str.	29.66	16.00	9.60	166.33	6,81
ery_1	28.00	17.66	9.66	166.66	6,06
ery_2	33,66	29.66	19.00	246.33	16.20
ery_3	29.83	17.00	11.00	174.66	6.82
kan_1	32.00	20,66	12.73	192.66	9.30
kan_2	25.50	13.00	2.00	165.00	6.45
kan_3	26.66	*	*	159.80	5.85
CD (0.05)	2.18	1.05	0.45	4.28	0.066

* No nodules

സംഗ്രഹം

തുവരയുടെ വേരുകളിൽ വളരുന്ന rararrairoT ക്ഷത്തിൽ നിന്ന് പാക്യജനകം സംരേ
ക്കുന്ന റൈസോബിയം എന്ന ബാക്ടീരിയയിൽ സ്ട്രെപ്ടോമൈസിൻ, എറിത്രോമൈസിൻ,
കനാമൈസിൻ എന്നീ ആൻറിബയോട്ടിക്കുകംക്കെതിരെയുള്ള ഒൻപതു തരം ബാക്ടീരിയക
ളെ വേർതിരിച്ചെടുക്കുകയും, അവ എത്രമാത്രം പാക്യജനകം ബഹാർ എന്നയിനം തുവരയിൽ
സംരേിക്കുന്നു എന്ന് ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് അഗ്രിക്കൾച്ചറൽ സയൻസസ്, ബനാറസ്
ഹിന്ദു സർവകലാശാലയിൽ പരീക്ഷണത്തിന് വിധേയമാക്കി. എറിത്രോമൈസിൻ, കനാ
മൈസിൻ എന്നീ ആൻറിബയോട്ടിക്കുകംക്കെതിരെ പ്രതിരോധശക്തിയുള്ള രണ്ടുതരം റൈ
സോബിയം ബാക്ടീരിയകളും സാധാരണ രീതിയിൽ പാക്യജനകംസംരേിക്കുന്നു എന്നും, കനാ
മൈസിൻ പ്രതിരോധശക്തിയുള്ള റൈസോബിയം വേരുകളിൽ മൂലാർബുദങ്ങളം ഉണ്ടാക്കു
വാൻശേഷി നശിച്ചതായും ബാക്കിയുള്ളവ സാധാരണ രീതിയിൽ നിന്ന് താഴ്ന്ന നിലവാര
ത്തിൽ പാക്യജനകം സംരേിക്കുന്നതായും തെളിഞ്ഞു.

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