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Short Communication

Symbiotic behaviour of phage-resistant mutants of pigeonpea [*Cajanus cajan* (L.) cv. Bahar] rhizobial strain IHP-195 with the main and alternate hosts

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The phage-resistant (V^r) mutants of an effective rhizobial strain do not retain the symbiotic property as their parent and become either ineffective [1&2] or partially effective [3] with their original legume host. However, no study has been conducted till date to know the symbiotic behaviour of such mutants with the alternate legume hosts. The rhizobia nodulating pigeonpea belong to the "cowpea miscellany" group, which have a wide host range. In such a situation, curiosity therefore arose regarding the ecological significance of V^r mutants. This study was undertaken to investigate the nodulation efficiency and symbiotic effectiveness of V^r mutants of pigeonpea rhizobial strain IHP195 with one cultivar each of the original (main) host and five alternate hosts to determine the change in host range nodulation capacity, if any, of a rhizobial strain due to its mutation for phage resistance.

The rhizobial strains used in this study included strain IHP 195, one of its streptomycin-resistant (Sm^r) mutants (IHP 195 Sm^r-1) and seven phage-resistant (V^r) mutants. All the V^r mutants were derived from single Sm^r mutant, IHP 195 Sm^r-1. The streptomycin- as well as phage-resistant mutants were of spontaneous origin, which were isolated on specific (Yeast-mannitol-agar) media carrying lethal concentration of streptomycin (200 $\mu g.ml^{-1}$) and/or high titre phage (10^8 plaque forming units (PFU) ml^{-1}). The legume hosts taken for determination of symbiotic effectiveness of rhizobial strains included one cultivar each of main host, i.e. pigeonpea [*Cajanus cajan* (L.) cv. Bahar] and five alternate hosts, *viz.* mungbean *(Vigna radiata* L. cv. HUM-1), urdbean (*Vigna mungo* L. cv. T-9), cowpea (*Vigna unguiculata* L. cv. Pusa Barsati), soybean (*Glycine max* L. cv. JS-335) and siratro (*Macroptilium atropurpureum* L. Wild type). Seeds of different legume hosts were surface-sterilized with 0.2% acidified mercuric chloride for 3-5 minutes (depending on seed size) and then thoroughly rinsed for 4-5 minutes with sterile distilled water [4]. Surface-sterilized seeds were germinated inside petridish containing water agar (1% w/v). Following germination (1 cm long radicle), the seedcoats were removed carefully with forceps and seedlings were transferred to nutrient agar slants (Thornton's medium) in glass test tubes (200 × 25 mm for mungbean, urdbean and siratro; and 200 × 38 mm for pigeonpea, soybean and cowpea). Rhizobial suspension (0.5 ml, containing approx. 10^8 cells) was inoculated into each tube after 24 hours of seedling growth (when the primary root from radicle attains 4-8 cm length). The plants were grown in a culture room at 26±2°C under fluorescent light (intensity of 3 K lux) with 14/10-hour light/dark cycle. The experiment was laid out in completely randomized design with four replications. The symbiotic parameters chosen for observation were nodule number and fresh weight, nitrogenase activity [5] and plant dry weight after 45 days of inoculation (DAI). Duncan's multiple range tests were performed for comparing different treatment means [6].

With respect to nodule number and fresh weight as well as nitrogenase activity (μM C_2H_2 reduced h^{-1} $plant^{-1}$), the performance of the streptomycin-resistant (Sm^r) mutant, IHP 195 Sm^r-1 was superior to the parental strain IHP 195 in case of cowpea, soybean and siratro but inferior to the parental strain in case of pigeonpea, mungbean and urdbean (Table 1). The plant biomass (including dry weight of shoot, root and nodule) gained at 45 DAI in different treatments of legume hosts was found to be in accordance with the result of other symbiotic parameters. Among the phage-resistant (V^r) mutants, IHP 195 Sm^r-1V^r-6 performed well with all hosts in general, while IHP 195 Sm^r-1V^r-3 significantly superseded other strains (including the parent and Sm^r mutant) with only host, soybean. Three V mutants (IHP 195 Sm^r-1V^r-3, V^r-5 and V^r-7) failed to nodulate mungbean while V^r-3 and V^r-5 in case of urdbean and only V^r-5 in case of

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Table 1. Plant biomass* (mg. plant^{-1}) of legume hosts in association with pigeonpea-rhizobial strain IHP-195 and its mutants

Rhizobial strain	Mungbean (cv.HUM-1)	Urdbean (cv. T-9)	Cowpea (cv. Pusa Barsati)	Soybean (cv.JS-335)	Siratro (Wild type)	Pigeonpea (cv. Bahar)
Uninoculated	215c**	170fg	1000d	380d	90e	340e
	(0.0,0.0,0.0)***	(0.0,0.0,0.0)	(0.0,0.0,0.0)	(0.0,0.0,0.0)	(0.0,0.0,0.0)	(0.0,0.0,0.0)
IHP 195 (Parent)	510a	310a	1250b	400cd	205b	990a
	(16.5,90.0,5.1)	(20.0,50.0,3.5)	(14.0',50.0,3.2)	(1.0,3.0,0.3)	(7.5,20.0,3.2)	(29.0,390.0,7.3)
IHP 195 Sm^r-1	415b	280b	1550a	435b	250a	820b
	(5.0,32.5,2.5)	(15.0,42.0,3.0)	(13.0,150.0,6.5)	(2.0,7.0,0.7)	(9.0,25.0,4.5)	(31.0,325.0,7.10)
IHP 195 Sm^r-1 V^r-1	225c	180efg	1075c	410cd	105de	395de
	(1.0,6.0,0.5)	(0.5,1.0,0.2)	(2.5,20.0,1.2)	(2.0,5.0,0.6)	(1.5,4.5,0.8)	(5.0,55.0,1.52)
IHP 195 Sm^r-1 V^r-2	210c	195def	1100c	400cd	100de	430de
	(0.5,5.0,0.4)	(1.5,2.0,0.3)	(1.5,10.0,0.8)	(2.5,10.0,0.9)	(0.5,2.0,0.5)	(10.0.100.0,1.95)
IHP 195 Sm^r-1 V^r-3	195c	210d	1260b	580a	125d	375de
	(0.0,0.0,0.0)	(0.0,0.0,0.0)	(7.0,100.0,5.0)	(3.0,18.0,1.2)	(4.0,12.0,1.8)	(4.0,75.0,1.30)
IHP 195 Sm^r-1 V^r-4	205c	200de	1080c	430c	95e	400de
	(0.5,3.0,0.3)	(3.0,5.0,0.5)	(1.5,15.0,1.0)	(0.5,1.5,0.2)	(0.5,1.5,0.3)	(5.0,80.0,1.80)
IHP 195 Sm^r-1 V^r-5	200c	165g	975d	390d	120de	365de
	(0.0,0.0,0.0)	(0.0,0.0,0.0)	(0.0,0.0,0.0)	(0.5,1.5,0.1)	(2.0,8.0,1.2)	(0.0,0.0,0.0)
IHP 195 Sm^r-1 V^r-6	245c	240c	1225b	425c	175c	610c
	(2.5,15.0,1.1)	(10.0,16.0,1.2)	(12.0,50.0,3.0)	(1.0,3.0,0.3)	(7.0,20.0,3.1)	(12.0,130.0,2.90)
IHP 195 Sm^r-1 V^r-7	210c	190defg	1110c	410cd	125d	495cd
	(0.0,0.0,0.0)	(3.0,2.0,0.3)	(1.0,5.0,0.5)	(5.0,12.0,0.8)	(1.5,6.0,1.2)	(7.0,75.0,1.75)
Mean	263	214	1162.5	426	139	522
SEM	12.75	6.19	13.69	7.50	7.07	33.49

*Plant biomass includes dry weight of shoot, root and nodule; **Means within a column having at least one common letter are not significantly different according to Duncan's test; ***Figures in parentheses indicate nodulation (nodules plant^{-1}), nodule fresh weight (mg.plant^{-1}) and nitrogenase activity (μM C_2H_2 reduced h^{-1} plant^{-1}), respectively.

pigeonpea and cowpea were also found totally ineffective. Nodule appearance was delayed by 2 to 8 days in pigeonpea, mungbean, urdbean and cowpea due to inoculation with V^r mutants.

In case of soybean, most of the V^r mutants exhibited earlier nodulation (3 to 4 days earlier) as well as higher symbiotic effectiveness as compared to the parental strain. This differential behavior of V^r mutants with soybean partly supports the findings of Gottfert *et al.* [7] that *nod* VW rhizobial mutants did not behave in the same manner with soybean as in case of mungbean, cowpea and siratro.

With siratro, the test legume host for *Bradyrhizobium*, none of the V^r mutants were found ineffective but all of them were inferior to IHP 195 as well as IHP 195 Sm^r-1. However, the performance of V^r-6 was satisfactory, though statistically not at par with IHP 195. The mutant V^r-3 was also moderately effective. Nodules appeared in 10 days due to inoculation with IHP 195 and IHP 195 Sm^r-1 but in 12-15 days with V^r mutants.

The study on interaction of drug resistant marked V^r mutants with host legumes widens our idea on the effectiveness of these mutants. Modification of host range nodulation capacity of the rhizobial strains after their mutation for phage-resistance is likely to be overlooked in field experiments but has great significance in ecological studies.

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