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Original Research Article

Interaction between *Rhizobium leguminosarum* strains and different varieties of pea

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A B S T R A C T

The present study was undertaken to obtain cultivar and region specific rhizobia for pea. Soil samples were collected and analysed for soil characteristics. Six isolates HC–1 to HC–6 for *Rhizobium* isolated from 3 different cultivars Arkel, Pb-88 and Matar Ageta-6 (MA-6) grown in soils of Hoshiarpur region were screened and tested for legume-*Rhizobium* symbiosis along with a reference culture with three varieties of pea at Bahuwal soil. In general, all the *Rhizobium* strains were found to improve most of the symbiotic parameters. Reference culture (P-4) interacted the most with cultivar Arkel and Matar Ageta-6 (MA-6) for nodulation, leghaemoglobin content, nitrogenase activity and grain yield whereas HC-2 was found best with Pb-88 for plant dry weight and nitrogen uptake.

K e y w o r d s

Rhizobium, Pea *(Pisum sativum L.)*, Strain X cultivar X soil interaction

Introduction

Biological nitrogen fixation, a microbiological process, which converts atmospheric nitrogen into plant usable form offers economically and ecologically sound means of reducing external input and improving internal N in the soil. Of all the nitrogen-fixing microorganisms, *Rhizobium* has the maximum ability to fix nitrogen in association with legume crops, a part of which is made available to host and remainder benefits the succeeding crops (Tyerman *et al.,* 1995; Tariq *et al.,* 2014). The recognition of *Rhizobium* by a legume seems to be mediated by protein (lectin) that links the bacterium to the root hair. The binding sites for this protein in the cell wall of plant and in the bacterial capsules are antigenically related, presumably they are also similar in structure. Legume-*Rhizobium* symbiosis is the most promising plant bacterium association for immediate increase in grain yield through biological nitrogen fixation (Verma *et al.,* 1998; Gresshoff *et al.,* 2014). The efficiency of this association depends upon the establishment of strains which are efficient and are able to compete with native rhizobia. Rhizobial inoculation can increase the grain yield of most of the pulse crops to the tune of 10 to 15 per cent (Ali and Chandra, 1985). The origin of isolate from specific location and host varietal specificity has

been reported to influence the establishment and success of inoculation. It was therefore, of interest to develop cultivar and area specific strain of *Rhizobium* for Pea (*Pisum sativum* L.) to harness maximum use of this association to be grown in specific area of Hoshiarpur.

Material and Methods

Rhizobium leguminosarum strains were isolated from healthy nodules of different varieties of pea grown in soil samples collected from different regions of Hoshiarpur viz. Nawanshehar and Bahuwal. Seeds of pea *(Pisum sativum* L.*)* var. Arkel, Pb-88 and Matar Ageta-6 (MA-6) obtained from Department of Vegetable Crops, Punjab Agricultural University, Ludhiana were used in the present study.

The soil samples were analysed for clay, silt and sand (Bouyoucos, 1962), electrical conductivity (Richards, 1954), organic matter (Jackson, 1967) and total nitrogen (Black, 1965). The value of E.C. was 0. 350 mmhos/cm and 0.294 mmhos/cm in Bahuwal and Nawanshehar soils, respectively. The organic matter content was maximum in Nawanshehar (0.87%) while it was 0.84% in Bahuwal soils (Table 1).

A pot experiment was conducted by taking the unsterilized soils from different locations of Hoshiarpur region. Seeds of three cultivars were sown in pots containing these soils. After 45 days of sowing, the plants were observed for number of nodules and dry weight and Rhizobial isolations made from nodules. Cultivar Arkel showed good nodulation and plant dry weight in soils of Bahuwal and Nawanshehar. *Rhizobium* isolations were made from each nodule (Table 2).

Gram staining was done to ensure purity and freedom from Gram-positive bacteria. The field experiment was conducted using the randomized block factorial design. This experiment was performed in Bahuwal soil using three important cultivars (Arkel, Pb-88 and MA-6) and six strains (HC-1, HC-2, HC-3, HC-4, HC-5 and HC-6) of *Rhizobia* isolated from nodules of different cultivars of pea grown in various soils of Hoshiarpur (Nawanshehar and Bahuwal), along with a reference strain (*Rhizobium leguminosarum* P-4) obtained from Department of Microbiology, Punjab Agricultural University, Ludhiana. The seeds were treated with charcoal based *Rhizobium* cultures using standard method (Vincent, 1970). Uninoculated seeds served as control. The seeds were sown in rows with a distance of 30 cm with a plant-to-plant distance of 10 cm with a plot size of 4m X 2m. Observations for nodulation, shoot dry weight, leghaemoglobin (Lb) content (Wilson and Reisenauer, 1963), nitrogenase activity (Hardy *et al.,* 1973) and total nitrogen content (Mckenzie and Wallace, 1954) were taken after 45 days of sowing. The grain yield was recorded at harvest.

Results and Discussion

All the six isolates were tested along with reference strain (P-4) to study their effect on various symbiotic parameters in three varieties of pea.

All the *Rhizobium* cultures resulted in significant increase in nodulation compared to uninoculated control. Among the cultures used, P-4 and HC-6 were at par amongst them, but were significantly better than all other cultures. Maximum nodulation was observed in Pb-88, which is significantly better than other two cultivars. Culture P-4 gave significantly higher nodules on cultivars Arkel and MA-6 whereas HC-6 with cultivar Pb-88 (Table 3).

Inoculation of *Rhizobium* strains significantly enhanced the fresh weight of nodules compared to uninoculated control. Similar to the pattern of nodule number per plant, nodule fresh weight was found to be maximum in P-4 and HC-6 and was significantly better than other cultures. Amongst cultivars Arkel represented highest fresh weight of nodules followed by Pb-88 and it was significantly better than that of MA-6. Cultivars Arkel and Pb-88 showed good interaction with all isolates but the response of MA-6 to isolates HC-3, HC-4 and HC-5 was found to be poor. Reference culture P-4 interacted the most with cultivar Arkel followed by Pb-88 and MA-6 (Table 4).

The dry matter accumulation was found significantly better in plants inoculated with *Rhizobium* compared to uninoculated control. Among the *Rhizobium* cultures, P-4 followed by HC-6 and HC-2 resulted in maximum accumulation of plant material and were significantly better than all other isolates. Variety Pb-88 showed highest plant dry weight accumulation followed by var. Arkel and MA-6. Isolates HC-6 and P-4 represented the best interaction with Arkel while HC-2 with Pb-88 (Table 5).

Lb content of the nodules is taken as the index of nodule efficiency as it regulates the oxygen supply to the bacteroid and hence the nitrogenase activity. Lb content of nodules produced by introduced *Rhizobium* isolates was found to be significantly high compared to uninoculated control. The nodules formed by HC-2 followed by P-4 and HC-1 showed maximum Lb content and it were significantly higher than all other strains. This content was also found to be controlled by host genotypes as all the three varieties differed significantly for this character. Cultivar Pb-88 represented highest Lb content, followed by Arkel and it was significantly better than that of MA-6. *Rhizobium* isolates HC-1, HC-2 and HC-5 interacted best with Pb-88 while P-4 with Arkel (Table 6).

Nitrogenase activity was found to be significantly improved with *Rhizobium* inoculation. The strain P-4 and HC-4 were at par amongst them and represented highest nitrogenase activity. Cultivars were found to exhibit significant variation in their potentials to reduce nitrogen as they differed significantly for acetylene reduction activity. Cultivar Arkel represented maximum nitrogenase activity followed by Pb-88 and MA-6. HC-4 and P-4 interacted best with Arkel (Table 7).

The nitrogen content of the whole plant is the sum total of the nitrogen uptaken from the soil and that fixed by nodules from atmosphere. Nitrogen uptake of plants was significantly improved with *Rhizobium* inoculation. Isolates P-4, HC-6 and HC-2 represented highest nitrogen uptake and were at par amongst them. Cultivar Pb-88 showed maximum N uptake, followed by Arkel aand these two cultivars were better than that of MA-6. Isolate HC-6 interacted best with Arkel and it was at par with reference culture for this cultivar (Table 8).

The yield was found significantly more in plants inoculated with *Rhizobium* cultures compared to control. *Rhizobium* isolates P-4 and HC-2 were at par amongst themselves and were significantly better than other isolates. Cultivars differed significantly for their potentials for grain yield. Arkel showed highest yield followed by MA-6 and Pb-88 respectively. Isolate P-4 interacted best with Arkel and MA-6 while HC-2 interacted best with Pb-88 (Table 9).

Better nodulation due to *Rhizobium* inoculation indicates poor status of native rhizobia and /or better competitive ability of the introduced strains for nodule formation (Fobert *et al.,* 1991, Indian Agriculture Research Institute., 2003). The increased amount of Lb content in inoculated plants reflects the efficiency of *Rhizobium* isolates to help the plants to synthesize more Lb (Klucas *et al.,* 1985; Tariq *et al.,* 2014). Due to increased nitrogenase activity there is more fixation of nitrogen which helps in enhancing photosynthetic efficiency of plant and hence there is more accumulation of dry matter in plants inoculated with *Rhizobium.* Our results are in consonance with the studies of Kanaujia *et al.* (1999) and Santalla *et al.* (2001). The improved nodulation and nitrogenase activity resulted in increased nitrogen content of plants inoculated with *Rhizobium.* The increase in grain yield due to *Rhizobium* inoculation with all the strains was attributed to their superiority over native *Rhizobia*. Buttery *et al* (1990) found that seed inoculation with *Rhizobium* strain increased nodulation, final dry matter and yield.

The different strains have been found to behave differently for different varieties indicating a need to develop cultivar specific rhizobial strain. Inoculation of *Rhizobium* strains certainly improved growth and nitrogen fixation, which may help in enhancing the yield potential of this crop.

Table.1 Physicochemical properties of soils

Location	**Soil Type**	**Clay (%)**	**Silt (%)**	**Sand (%)**	**E.C. (mmhos/cm)**	**Organic C (%)**	**Organic matter (%)**	**Total N (%)**
Nawan Shehar	Sandy loam	14.81	31.8	53.4	0.294	0.502	0.87	0.04
Bahuwal	Sandy loam	12.80	27.8	59.4	0.350	0.487	0.84	0.04

Table.2 Nodulation and plant dry weight of three cultivars of Pea in different soils and details of *Rhizobium* isolates

Location	Cultivars								
	Arkel			Pb-88			MA-6		
	Nodule number/ plant	Plant dry wt. (mg)	Isolates	Nodule number/ plant	Plant dry wt. (mg)	Isolates	Nodule number /plant	Plant dry wt. (mg)	Isolates
Nawan Shehar	20	230	HC-1	4	250	HC-2	6	170	HC-3
Bahuwal	17	140	HC-4	5	280	HC-5	19	180	HC-6

Table.3 Effect of inoculation of *Rhizobium* isolates on nodule number/plant

Rhizobium isolates	Varieties			
	Arkel	MA-6	Pb-88	Mean
Control	23.3	9.3	10.3	14.3
HC-1	33.3	44.0	67.3	48.2
HC-2	52.3	10.0	68.3	43.5
HC-3	42.3	33.3	57.3	44.3
HC-4	46.3	37.3	72.6	52.1
HC-5	44.3	42.3	62.3	49.6
HC-6	55.6	49.3	60.6	55.2
P-4	68.3	73.6	57.3	66.4
Mean	45.7	37.4	57.0	

CD (5%) Varieties = 0.788, Cultures = 1.28, Varieties X cultures = 2.23

Table.4 Effect of inoculation of *Rhizobium* isolates on fresh weight of nodules (mg)/plant

Rhizobium isolates	Varieties			
	Arkel	MA-6	Pb-88	Mean
Control	140	103	153	132
HC-1	260	166	263	230
HC-2	410	153	303	288
HC-3	263	96	193	184
HC-4	283	110	293	228
HC-5	263	96	273	211
HC-6	403	233	296	311
P-4	453	326	330	370
Mean	309	160	263	

CD (5%) Varieties = 11.30, Cultures = 18.46, Varieties X cultures = 31.98

Table.5 Effect of inoculation of *Rhizobium* isolates on dry weight (mg)/plant

Rhizobium isolates	Varieties			
	Arkel	MA-6	Pb-88	Mean
Control	70	93	96	86
HC-1	193	190	386	256
HC-2	313	196	410	306
HC-3	293	156	103	184
HC-4	273	220	376	290
HC-5	196	176	293	222
HC-6	453	253	280	328
P-4	453	290	333	358
Mean	280	197	285	

CD (5%) Varieties = 8.35, Cultures = 13.64, Varieties X cultures = 23.64

Table.6 Effect of inoculation of *Rhizobium* isolates on leghaemoglobin (mg/g nodules)

Rhizobium isolates	Varieties			
	Arkel	MA-6	Pb-88	Mean
Control	0.393	0.426	0.960	0.593
HC-1	3.03	3.16	5.20	3.80
HC-2	4.96	0.393	6.96	4.10
HC-3	3.07	0.460	2.79	2.10
HC-4	3.12	2.06	2.77	2.65
HC-5	2.48	1.39	6.40	0.428
HC-6	4.40	2.15	1.84	2.80
P-4	5.59	2.94	2.92	3.82
Mean	3.38	1.62	3.73	

CD (5%) Varieties = 0.0316, Cultures = 0.051, Varieties X cultures = 0.089

Table.7 Effect of inoculation of *Rhizobium* isolates on nitrogenase activity (mM C_2H_2 reduced/plant)

Rhizobium isolates	Varieties			
	Arkel	MA-6	Pb-88	Mean
Control	65	31	32	43
HC-1	150	54	123	109
HC-2	180	32	125	112
HC-3	152	45	53	83
HC-4	201	35	154	130
HC-5	157	67	93	106
HC-6	220	71	51	114
P-4	255	92	73	140
Mean	172	53	88	

CD (5%) Varieties = 5.62, Cultures = 9.18, Varieties X cultures = 15.9

Table.8 Effect of inoculation of *Rhizobium* isolates on nitrogen uptake (mg)/plant

Rhizobium isolates	Varieties			
	Arkel	MA-6	Pb-88	Mean
Control	1.26	1.50	1.78	1.52
HC-1	3.66	4.89	9.10	5.88
HC-2	9.59	4.63	12.7	8.97
HC-3	5.72	2.55	2.16	3.48
HC-4	5.82	4.95	10.9	7.22
HC-5	4.17	2.86	6.75	4.59
HC-6	13.1	7.08	7.02	9.07
P-4	13.5	8.39	10.6	10.8
Mean	7.11	4.61	7.63	

CD (5%) Varieties = 0.208, Cultures = 0.341, Varieties X cultures = 0.591

Table.9 Effect of inoculation of *Rhizobium* isolates on grain yield (g)/plot

Rhizobium isolates	Varieties			
	Arkel	MA-6	Pb-88	Mean
Control	312	296	312	307
HC-1	486	482	490	486
HC-2	767	814	822	801
HC-3	532	514	509	518
HC-4	570	495	477	514
HC-5	604	661	565	610
HC-6	612	612	621	615
P-4	1340	1240	940	1173
Mean	653	639	592	

CD (5%) Varieties = 18.6, Cultures = 30.3, Varieties X cultures = 52.6

Table.10 Correlation matrix of different symbiotic parameters

	Nodule number	Fresh weight of nodules	Dry weight of plant	Lb Content	Nitrogenase activity	Nitrogen uptake	Grain yield
Nodule number	-						
Fresh weight of nodules	0.693						
Dry weight of plant	0.758	0.821					
Lb Content	0.729	0.719	0.765				
Nitrogenase activity	0.489	0.804	0.702	0.621			
Nitrogen uptake	0.751	0.847	0.968	0.741	0.647		
Grain yield	0.528	0.622	0.572	0.407	0.387	0.630	-

Critical value of r at 5% = 0.34

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