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Full Length Research Paper

Symbiotic effectiveness of pea-rhizobia associations and the implications for farming systems in the western Loess Plateau, China

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Interactions between pea (*Pisum sativa* L.) cultivars and *Rhizobium* strain affect the symbiotic relationship and ultimately both the nitrogen fixing capacity and the yield. Since *Pisum sativum* L. is poorly nodulated in the Loess Plateau of China where this crop is grown, the response of pea cultivars Yannong No.2 and Luwan to inoculation with reference *Rhizobium* ACCC16101, ACCC16103 and selected strains, namely SY3.1, SY12, GWW16, GWC7.3, GDB27, GQW28, GQZ5, GLC1, QGW19 and XC3.1, was studied in two soil types in a greenhouse. Selected strains were isolated from the root nodules of pea (*Pisum sativum* L.), broad bean (*Vicia faba* L.) and lentil (*Lens culinaris* L.) plants in the Loess Plateau of China. Analyses focused on the nodule number, nodule dry weight, plant dry weight, nitrogenase activity, total N accumulation of per plant and seed yield. A significant interaction between pea and rhizobia was observed. The selected strain GDB27 was considered to be the best symbiotically efficient for all pea cultivars in the various soils. Strain XC3.1 evidenced relatively superior symbiotic effectiveness with pea cultivar Yannong No.2. Strains QGW19 and GWW16 performed well with Luwan. Correlation among the parameters for the plants showed that N_2-fixation was positively and strongly correlated with nodule dry weight, whole plant dry weight and Acetylene Reduction Assay (ARA) in both soils, but not always significantly correlated with nodule number. Yield increases will follow widespread adoption of inoculation with cultivar-specific strains of *Rhizobium*.

Key words: Nitrogen fixation, *Rhizobium*, Loess Plateau, soil type, yield increase

INTRODUCTION

Pea (*Pisum sativum L.*) is an important rotation crop in many arid and semi-arid parts of the world (Drevon et al., 2001; Soon et al., 2004), the nitrogen benefits to the following crop are reported (Graham and Vance, 2003; Bourion et al., 2007; Talukder et al., 2008). As a better N-fixing plant, nodulated pea can fix about 75 kg/ha N (Sun and Liu, 2000). Therefore, pea cultivation in arid and semi-arid agricultural regions is of great significance to reducing the need for chemical fertilizers, protecting the ecological environment and progressing the cause of sustainable agricultural development.

There is no doubt that the use of effective rhizobial strains as inoculants will ensure effective nodulation (Gwata et al., 2003; Tahir et al., 2009), but to maximize nitrogen fixation, a successful inoculation is highly dependent on strain by cultivar interactions (Kiers et al., 2007; Oğutcu et al., 2008; Bourion et al., 2010). Significant effects of rhizobial strain and of strain×pea cultivar interactions on nodule and shoot biomass or N acquisition have been reported under controlled and field conditions (Fezenko et al.,1995; Martensson and Rydberg, 1996; Mia et al., 2010). There is often a large population of the indigenous rhizobial population in the soil that often include ineffective or low nitrogen-fixing efficiency strains. Therefore, a better way to enhance rhizobial effectiveness is to select superior strains with proven nitrogen-fixing

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ability specific to the pea cultivar and one that is highly effective and competitive against the indigenous strains.

The *Rhizobium* in northwest of China live in a soil environment with frequent drought, alkalinity and high salt concentration (Guo and He, 2009). Nitrogen fixation in many croplands depends on less efficient indigenous rhizobia and a low level of symbiosis with the host plant, which limit the potential to fix nitrogen (Chen, 2004).

The work reported here was in the context of the Australian Center for Agricultural Research (ACIAR) supported project aimed *inter alia* at enhancing legume production for the western Loess Plateau of China. Thus, the direct aims of the work presented in this paper are: (i) to select effective symbiotic associations from arid and semi-arid regions in the Loess Plateau of China, (ii) to investigate responses to inoculation with rhizobium in two soils in a greenhouse. These were assessed by determining the symbiotic effectiveness in different pea–rhizobia associations in two different soil types.

Materials and Methods

Bacterial strains and Plant material

Reference *Rhizobium* strains ACCC16101, ACCC16103 obtained from the Agricultural Culture Collection of China were compared with 10 selected strains that were previously isolated from the different ecological area in the Loess Plateau of China. The origin of each strain is shown in Table 1. The selected strains were isolated from peas and other host plants which belong to the cross-inoculation group. The two drought-tolerant cultivars commonly grown in middle area of Gansu, Yannong No.2 and Luwan were used.

Soil

Two soil types, which constitute the major soil in the middle of Gansu, were compared in pot experiments to select suitable strains for different peas cultivars in different soils. Soil 1 (loessial soil) was obtained from cropland near Lanzhou city. Soil 2 (irrigation-silting soil) was obtained from the experimental station of the Gansu Academy of Agricultural Sciences. Samples of the soils (removed litter and top 3-5 mm of soil) were air dried and ground to pass through a 5-mm sieve. The main soil analysis determinations of the two soils are shown in Table 2.

Inoculum preparation

Inoculants were prepared by growing the bacterial strains to late exponential phase[Optical Density at 620 nm(OD_{620})=0.8-0.9] (Haythem et al., 2008) in 250 ml Erlenmeyer flasks containing 100 ml of yeast mannitol broth (YMB) and incubated at 28 ℃ for 2 days on a rotary shaker at 160 rpm(Prevost et al., 1987b). Pea seeds were surface sterilized by treatment with 95 % ethanol for 5 min and then transferred into $HgCl_2$ solution of 0.1 % for 5 min, and rinsed 6 times with sterile water. Then surface-sterilized pea seeds were treated with the rhizobial suspension (Somasegaran and Hoben, 1985) for 30 min and immediately sown in clay pots.

Pot experiments

The pot experiments were performed in two separate experiments respectively in Soil 1 and Soil 2 in a greenhouse in order to test the symbiotic effectiveness and stability of the strains when inoculated into two pea cultivars, Yannong No.2 and Luwan. The experiments were carried out with 12 strains inoculated onto Yannong No.2 and Luwan respectively in clay pots (diameter 30 cm) filled with 15 kg soil samples of Soil 1 and Soil 2. The experiments were performed in a completely randomized block design with six replicates, and one treatment was used as control (CK- without inoculation). 0.15 g P_2O_5 (calcium superphosphate) per kilogram soil and 0.15 g K_2O (K_2SO_4) per kilogram soil was applied. Inoculated pea seeds were sown in each pot and 10 ml liquid culture containing rhizobium was poured into pots in order to ensure the inoculation effect. After emergence five plants were left per pot.

Data collection

For the determination of nodule number and dry weight, whole plant dry weight and nitrogen activity and nitrogen content, three replicates per treatment were used at full flowering stage. Three replicates were used for seed yield.

At full flowering stage, two plants from per pot for ARA and other three plants were used for other measurments. Plant shoot and root fractions on each plant were separated, and the nodules were severed from the roots to dry at 60 ℃ for 48 h. Nitrogen concentration was analyzed using the Kjeldahl method (Bremner and Breitenbeck, 1983a) and total nitrogen content (whole plant dry weight×N concentration) per plant, and the N fixed (plant N content in inoculated pots-plant N content in uninoculated pots) were calculated (Oğutcu et al., 2008).

The determination of nitrogenase activities

Nitrogenase activities of rhizobium was determined by Acetylene Reduction Assay (ARA) (Hardy et al., 1968) using gas chromatography (Trace GC2000, Chrom-cord, Italy). ARA measure was performed at the flowering stage. Nodule-bearing roots were incubated in 10 % C_2H_2 atmosphere. After 60 min of incubation, the ethylene formation rate was measured using gaseous phase chromatography. Three replicates of 0.5 ml gas samples were withdrawn from the root atmosphere of each plant, and ethylene production was determined. Pure acetylene and ethylene were used as internal standards (Mhadhbi et al., 2008).

Statistical analysis

Treatments were arranged in a randomized block design with six replications. Data were subjected to analysis of variance and the rhizobial strain, the plant cultivar and their interaction into the variance of analyzed parameters were determined. Analysis of variance (ANOVA) was performed using SPSS statistical software. When the F-value of the ANOVA was significant at the $P<0.05$ level of probability, Duncan's multiple comparison test was performed.

Results

Effect of inoculation on growth of pea in Soil 1 and Soil 2

Table 3 shows that the whole plant dry weight were affected by both rhizobium and cultivar, and the interaction between both factors in both soils at flowering stage. The inoculation with all strains induced a higher plant growth than un-inoculated control (CK) for two pea cultivars in soil 1. Plants inoculated with GDB27 for Yannong No.2 and with SY3.1 for Luwan produced the highest whole plant dry weight, respectively 1.7 and 1.8 fold higher than CK (Table 8). In soil 2, the highest whole

Table 1. Rhizobial strain used in this study.

Strains	Host plant	Source
SY3.1	pea	Yanan in Shanxi
SY12	pea	Yanan in Shanxi
GWW16	pea	Wuwei in Gansu
GWC7.3	broadbean	Wuwei in Gansu
GDB27	lentil	Dingxi in Gansu
GQW28	pea	Qingyang in Gansu
GQZ5	pea	Qingyang in Gansu
GLC1	broadbean	Linxia in Gansu
QGW19	pea	Geermu in Qinghai
XC3.1	pea	Lanzhou in Gansu
ACCC16101	pea	ACCC[1]
ACCC16103	pea	ACCC

[1]Agricuture Culture Collection of China.

Table 2. Chemical and physical analyses of soils in experiments.

Soils	Soil 1	Soil 2
Total N (g/kg)	0.52	0.64
Total P (g/kg)	0.58	0.75
Total K (g/kg)	15.44	18.41
$NH^{4+}N$ (mg/kg)	0.89	1.24
$NO^{3-}N$ (mg/kg)	4.57	9.46
Available P (mg/kg)	40.0	40.0
Available K(mg/kg)	136.1	156.0
Organic matter (g/kg)	14.0	16.8
pH	7.7	7.5

Table 3 Analysis of variance for whole plant dry weight in Yannong No.2 and Luwan inoculated with selected strains SY3.1, SY12, GWW16, GWC7.3, GDB27, GQW28, GQZ5, GLC1, QGW19, XC3.1 and reference strains ACCC16101, ACCC16103 in Soil 1 and Soil 2

Source of variation	Soil 1 Whole plant dry weight	Soil 2 Whole plant dry weight
Cultivar	[S]88.93 * * *	23.10 * * *
Rhizobia	2.55 *	2.89 * *
Cultivar×rhizobia	2.14 *	2.23 *

[S] *F*-values, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, ns: not significant

plant dry weight was also observed with GDB27 for two cultivars than CK (respectively 2.14 and 1.92 g/plant) (Table 9).

Effect of inoculation on nodulation in Soil 1 and Soil 2

The statistical analysis summarized in Table 4 showed that nodule number and nodule weight was affected by both rhizobium and cultivars, and the interaction between those two factors ($p \leq 0.001$) in soil 1 and soil 2. Inoculation increased the nodule number and nodule weight significantly in both soils. However, a apparent intraspecific variability was observed (Table 8), in soil 1, plants inoculated with GDB27 for Yannong No.2 and with QGW19 for Luwan produced the highest nodule number (respectively 34 and 33.8 nods/plant). GDB27 when inoculated onto two pea cultivars gave the highest nodule weight (respectively 70mg and 77.9mg per plant). During the second year (Table 9), the experiment was repeated in

Table 4 Analysis of variance for nodule number and nodule dry weight in Yannong No.2 and Luwan inoculated with selected strains SY3.1, SY12, GWW16, GWC7.3, GDB27, GQW28, GQZ5, GLC1, QGW19,XC3.1 and reference strains ACCC16101, ACCC16103 in Soil 1 and Soil 2

Source of variation	Soil 1		Soil 2	
	Nodule number	Nodule dry weight	Nodule number	Nodule dry weight
Cultivar	[S]32.5 * * *	326.10 * * *	9.89 * * *	123 * * *
Rhizobia	29.90 * * *	241.29 * * *	23.74 * * *	254.20 * * *
Cultivar×rhizobia	37.33 * * *	103.38 * * *	9.79 * * *	305.60 * * *

[S] *F*-values, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, ns: not significant

Table 5 Analysis of variance for total nitrogen content, N fixed and ARA in Yannong No.2 and Luwan inoculated with selected strains SY3.1, SY12, GWW16, GWC7.3, GDB27, GQW28, GQZ5, GLC1, QGW19, XC3.1 and reference strains ACCC16101, ACCC16103 in Soil 1 and Soil 2

Source of variation	Soil 1			Soil 2		
	Total N content	N fixed	ARA	Total N content	N fixed	ARA
Cultivar	[S]114.81 * * *	34.91 * * *	45.78 * * *	2.76ns	21.14 * * *	19.23 * * *
Rhizobia	9.38 * * *	10.58 * * *	9.84 * * *	10.96 * * *	11.25 * * *	11.44 * * *
Cultivar×rhizobia	3.96 * * *	4.46 * * *	4.24 * * *	7.64 * * *	7.85 * * *	6.89 * * *

[S] F-values, *** p≤ 0.001, ** p≤ 0.01, * p≤0.05, ns: not significant

different soil. The inoculation of Yannong No.2 with SY3.1 and the inoculation of Luwan with GDB27 induced more nodules than other treatments. Both nods/plant (25.8), and nodule weight (212.4mg and 163.6mg per plant respectively) was significantly higher with GDB27 than with other strains for the two cultivars. In both soils, the inoculation with QGW19 for Luwan also demonstrated a good symbiotic performance, nodule number (33.8 nod/plant and 20.3 nod/plant respectively) and nodule weight (70.6 mg/plant and 101.0mg/plant).

Effect of inoculation on N accumulation in Soil 1 and Soil 2

To assess the quantity of N fixed, the nitrogen content was measured in whole plants of Yannong No.2 and Luwan after inoculation with strains in soil 1 and soil 2 (Table 8, 9). In soil 1, the nitrogen content was affected by both rhizobium and cultivars, and the interaction between those two factors ($p \leq 0.001$) (Table 5). In soil 2, the nitrogen content was affected by rhizobia ($p \leq 0.001$) but not by cultivar, although the interaction between the two factors (rhizobium and cultivar) was statistically significant ($p \leq 0.001$) (Table 5). Then, the amount of N fixed was estimated in Tables 8, 9 as the difference between whole plant N in inoculated treatment and control. It was also affected by cultivar and rhizobium and the interaction between rhizobia and cultivar (Table 5). Generally, the inoculation induced a significant increase in N fixed compared to uninoculated plants, about 2-45 mg/plant (except for ACCC16103 inoculated Yannong No.2 in soil 1 and GLC1, GQZ5, SY3.1 inoculated Luwan in soil 2), and the selected strains GDB27, XC3.1, SY12 inoculated Yannong No.2, GDB27, QGW19, GWW16 inoculated Luwan induced on average 1-fold more N fixed than CK.

Effect of inoculation on ARA in Soil 1 and Soil 2

Table 5 shows that ARA was affected by both rhizobium and cultivar, and the interaction between both factors in both soils. *Rhizobium* inoculation significantly increased the ARA compared to the un-inoculated control (except GLC1 inoculated Yannong No.2 in soil 2). The maximum nitrogenase activity was expressed in both pea cultivars with association of strain GDB27 followed by cultivar Yannong No.2 with XC3.1 and cultivar Luwan with QGW19 and GWWW16. The nitrogen fixing ability of selected strains ranged from 1.56-20.51 µmol of C_2H_4

Table 6 Correlation Coeficients between total nitrogen content and nitrogen-fixing factors(nodule number, nodule dry weight, ARA)

Soil	cultivas	Nodule number	Nodule dry weight	ARA
Soil 1	Yannong No.2	0.6659 *	0.6535 *	0.7313 * *
	Luwan	0.5142	0.6587 *	0.8695 * *
Soil 2	Yannong No.2	0.3349	0.7156 * *	0.7761 * *
	Luwan	0.6785 *	0.6384 *	0.7625 * *

ARA: Acetylene Reduction Assay ** $p \leq 0.01$, * $p \leq 0.05$

produced $h^{-1}g^{-1}$ nodule fresh weight, whereas the reference strains ranged from 3.00-9.04 µmol of C_2H_4 produced $h^{-1}g^{-1}$ nodule fresh weight (Table 8, 9).

Correlation coefficients analysis of total nitrogen content and nitrogen-fixing factors

The results of correlation coefficient between total nitrogen content and nitrogen-fixing factors studied were shown in Table 6. The data show the nodule dry weight (r=0.67), ARA (r=0.78) were positively and significantly associated with total nitrogen content in both pea cultivars and soils, but not always significantly with nodule number. Obviously, the highest correlation coefficients found were with ARA.

Effect of inoculation on seed yield

Seed yields varied significantly between cultivars, rhizobium and soils. The inoculation generally increased significantly the seed yield for both cultivars in soil 1 (increased by 1.9%-67.3%), but in soil 2, only XC3.1, QGW19, GDB27, GWW16 inoculated both cultivars increased the seed yield compared to un-inoculated control, and in soil 2, the highest seed yield was observed for Yannong No.2 and Luwan with XC3.1 (respectively increased by 34.1% and 26.3%) (Table 8, 9). In soil 1 and soil 2, the seed yield was affected by both rhizobia and cultivar, and the interaction between those two factors ($p \leq 0.05$ and $p \leq 0.001$, respectively) (Table 7).

Discussion

The finding in this work of an efficient symbiotic interaction of Rhizobium with pea cultivars in different soils depended on rhizobia×cultivars interaction and the process of selection of efficient rhizobia. In our study, two pea cultivars and twelve strains showed cultivar×rhizobia interactions with respect to symbiotic performance (Table 8, 9), For example, SY3.1 for Yannong No.2, QGW19 and GWW16 for Luwan was more effective than other strains. This underlines the importance of such interaction when selecting a rhizobial inoculant. Some studies have also indicated the existence of variability in rhizobia×cultivar interactions (Santalla, 2001; Giller, 2001; Fall et al., 2003; Krasova-Wade and Bohlool, 2003). Namely the expression of the efficient symbiotic not only depends on simple contributions of symbiotic partners, but also includes rhizobia×cultivar interaction (Krasova-Wade and Bohlool, 2006; Mhadhbi et al., 2008).

Existing populations of soil Rhizobium will result in competition between inoculum strains and indigenous rhizobia for nutrients and living space (Li and Zhou 2002). This means the introduced strains have to compete with indigenous ones. However, the nitrogen-fixing efficiency and competitive ability of a strain are not necessarily correlated (Romdhane, 2007). In our study, GDB27 inoculated Yannong No.2 in the two soils gave the highest nodule number, nodule weight, ARA, whole plant dry weight and total nitrogen content, and SY3.1 inoculated Luwan in soil 1 also gave the highest whole plant dry weight and total nitrogen content, and XC3.1 inoculated Yannong No.2 in two soils and inoculated Luwan in soil 2 gave the highest seed yield, while QGW19 inoculated Luwan in soil 1 gave the highest seed yield. But the reference rhizobia, ACCC16101 and ACCC16103 (introduced from ACCC), which were previously found to establish an efficient symbiosis with local cultivar Yannong No.2 under field conditions (Xie et al., 2009) and the two rhizobium strains widely used in the field (Ning et al., 1997), didn't perform well in the two soils used in this experiment. This may be attributable to rhizobial competitiveness and soil environmental conditions. Competition for nodulation is a very complex and poorly understood phenomenon (Bogino et al., 2010). Many environmental conditions are limiting factors to the growth and activity of the N_2-fixing plants, including photosynthate deprivation, water stress, salinity, soil nitrate, temperature, heavy metals, and the biocides (Zahran, 1999). In our experiments, the symbiotic performance of strains inoculated pea cultivars was also observed in two soils (Table 8, 9). The Inoculation effect in Soil 1 is higher than Soil 2 in nodule number, N fixed and seed yield. This may be influenced by mineral nitrogen (NO_3^- in soil 2 is 2.07 times as much as that in soil 1). The process of nodulation may be promoted by relatively low levels of available nitrate, high concentrations depress nodulation

Table 8. Symbiotic performance of strains on pea cultivar Yannong No.2 and Luwan in Soil 1

Cultivars	Rhizobial strains	Nodule number plant^{-1}	Nodule dry weight (mg) plant^{-1}	ARA[a]	Whole plant dry weight (g) plant^{-1}	Total N content (mg) plant^{-1}	N fixed (mg) plant^{-1}	Seed yield (g) plant^{-1}
Yannong No.2	SY3.1	30.9a	18.1fgh	4.34e	1.48abc	48.78de	19.48de	1.35bc
	SY12	19.2de	20.8ef	6.77d	1.80ab	65.18ab	35.88ab	1.52ab
	GWWW16	26.7b	31.5c	3.05e	1.67abc	55.69bcd	26.39bcd	1.33bc
	GWC7.3	23.9bc	25.2d	3.30e	1.52abc	52.44cd	23.14cd	1.12c
	GDB27	34.0a	70.0a	11.15a	2.02a	70.28a	40.98a	1.62ab
	GQW28	23.4bc	24.3de	8.45bcd	1.45abc	44.78de	15.48de	1.49ab
	GQZ5	22.1cd	23.7de	9.54ab	1.94ab	63.50abc	34.20abc	1.61ab
	GLC1	25.5bc	41.0b	7.16cd	2.00a	64.66ab	35.36ab	1.67ab
	QGW19	15.2e	19.2fg	8.77bc	1.90ab	60.99abc	31.69abc	1.55ab
	XC3.1	17.1e	29.1c	10.95a	1.84ab	65.33ab	36.03ab	1.79a
	ACCC16101	9.5f	14.8h	4.25e	1.47abc	38.65ef	9.35ef	1.55ab
	ACCC6103	6.6f	16.6gh	4.12e	1.36bc	28.50f	-	1.56ab
	Uninoculated control	6.4f	8.3i	1.06f	1.21c	29.30f	-	1.07c
Luwan	SY3.1	22.8b	65.1d	11.42ab	1.36a	45.35a	24.15a	1.51ab
	SY12	12.7d	23.0h	3.32cd	0.96cde	32.66abcd	11.46bcd	1.35abc
	GWWW16	20.7bc	72.4b	10.92ab	1.32ab	43.10a	21.90ab	1.55a
	GWC7.3	8.4f	18.0i	3.23cd	1.23abc	38.57abc	17.37abc	1.33abc
	GDB27	23.2b	77.9a	13.26a	1.30ab	44.23a	23.03ab	1.55a
	GQW28	23.1b	69.8bc	8.99b	1.29ab	40.56ab	19.36ab	1.49abc
	GQZ5	21.7b	39.2g	5.66c	1.13abcd	38.39abc	17.19abc	1.44abc
	GLC1	16.1d	67.3cd	9.17b	1.22abc	39.36ab	18.16abc	1.26abc
	QGW19	33.8a	70.6bc	9.45b	1.31ab	43.09a	21.89ab	1.58a
	XC3.1	17.8cd	45.2f	4.01c	1.06bcd	36.53abc	15.33abcd	1.50abc
	ACCC16101	20.8bc	53.7e	3.00cd	0.88de	26.30cd	5.10d	1.06bc
	ACCC6103	21.9b	49.1f	3.05cd	1.00cde	28.59bcd	7.39cd	1.07bc
	Uninoculated control	7.7f	13.3j	0.98d	0.76e	21.20d	-	1.04c

[a]Values are given in umol C_2H_2 reduced hour^{-1} g^{-1}; Numbers are means of three replicates;
In each column, for each Rhizobium strain, values followed by the same letter do not differ significant at p = 0.05

Table 9. Symbiotic performance of strains on pea cultivar Yannong No.2 and Luwan in Soil

Cultivars	Rhizobial strains	Nodule number plant-1	Nodule dry weight (mg) plant-1	ARA[a]	Whole plant dry weight (g) plant^{-1}	Total N content (mg) plant^{-1}	N fixed (mg) plant^{-1}	Seed yield (g) plant-1
Yannong No.2	SY3.1	25.8a	58.0b	8.54bc	1.69bc	69.34bcd	21.70bcd	1.12de
	SY12	10.3gh	20.6e	9.06bc	1.94ab	82.58ab	34.94ab	1.20cd
	GWWW16	19.0cd	50.2c	3.69de	1.67bc	66.39bcde	18.76bcd	1.41bcd
	GWC7.3	7.3h	16.8e	3.24de	1.59bc	49.75ef	2.11d	1.37bcd
	GDB27	21.2bc	212.4a	20.51a	2.14a	93.12a	45.48a	1.49abc
	GQW28	15.8def	29.1d	5.24cde	1.73abc	69.68bcd	22.04bcd	1.41bcd
	GQZ5	12.0fg	25.2d	6.89bcd	1.82abc	55.25def	7.61d	0.87e
	GLC1	9.8gh	9.5f	1.56e	1.75abc	52.22def	4.58d	1.31bcd
	QGW19	18.0cde	26.2d	9.38bc	2.02ab	63.57cdef	15.93bcd	1.48abcd
	XC3.1	17.3cde	58.5b	10.11b	1.73abc	76.56abc	28.92abc	1.77a
	ACCC16101	14.8ef	29.1d	8.29bc	1.90abc	57.56def	9.92cd	1.55abc
	ACCC6103	23.0ab	28.9d	7.98bc	1.88abc	49.67ef	2.03d	1.60ab
	Uninoculated control	13.0fg	20.5e	1.89e	1.46c	47.64f	-	1.32bcd
Luwan	SY3.1	14.3cde	15.7hi	2.64de	1.37cd	37.68f	-	1.16bcde
	SY12	13.3de	27.8f	5.67cd	1.39bcd	56.99cde	13.17de	0.89fg
	GWWW16	18.0bc	32.5e	8.32bc	1.84a	78.12ab	34.30ab	1.34ab
	GWC7.3	11.0e	22.4g	2.43e	1.26d	52.64def	8.82def	1.04def
	GDB27	25.8a	163.6a	19.84a	1.92a	84.69ab	40.87a	1.32abc
	GQW28	11.7e	12.4i	3.08de	1.13d	48.66def	4.84efg	0.97efg
	GQZ5	13.3de	50.0c	8.04bc	1.16d	40.81f	-	1.06def
	GLC1	13.3de	19.2gh	2.23e	1.46bcd	42.29ef	-	0.83g
	QGW19	20.3b	101.0b	20.02a	1.73ab	81.25ab	37.43ab	1.49a
	XC3.1	16.8bcd	49.0c	8.11bc	1.69abc	62.57cd	18.75cd	1.49a
	ACCC16101	18.0bc	42.0d	7.43bc	1.43bcd	71.01bc	27.19bc	1.12cde
	ACCC6103	13.0de	40.5d	9.04b	1.82a	87.77a	43.95a	1.14bcde
	Uninoculated control	10.3e	7.2j	1.25e	1.46bcd	43.82ef	-	1.18bcd

[a]Values are given in μmol C2H2 reduced hour-1 g-1;Numbers are means of three replicates;
In each column, for each Rhizobium strain, values followed by the same letter do not differ significant at p = 0.05

(Eaglesham, 1989). Thus the selection of a strain not only must be considered a highly effective and competitive strain as inoculum (Hafeez et al., 2000; Naeem et al., 2004), but also considered the adaptation of the strain to regional soil environment (Chen et al., 2004).

A positive realtionship between N_2-fixation and nodule dry weight, ARA was observed, and we also found there was no always positive relationship between total N content and nodule number for any of the cultivars studied (Table 6). It may also indicate that the strain×cultivar interaction was a significantly factor in this study, since nodules from different cultivars had different specific activities (Table 5). Based on our results and similar such reports on symbiotic performances of N_2-fixing plants (Appunu and Dhar, 2006; Talukder et al., 2008), high nodule dry weight and ARA were generally considered as the trait of efficient symbiosis (Laguerre et al., 2007; Appunu et al., 2008).

In conclusion our results establish that the nitrogen fixed and seed yield of pea are increased in both soils with selected strain GDB27 inoculated onto Yannong No.2 and Luwan. This could be recommended to be the most promising rhizobia for pea. The symbiosis nitrogen efficiency of Yannong No.2 inoculated with XC3.1 is better than Luwan, whereas the symbiosis nitrogen efficiency of Luwan inoculated with QGW19 and GWW16 is better than Yannong No.2. Our inoculation trials substantiate the need for inoculating pea with efficient strains. The further comparisons of the selected strains×local varieties symbiosis is now needed among the cultivars and fields of the region, and further exploration of the interaction between the diversity of rhizobia and various cultivars of pea is needed. Expanded acquisition of information is needed on rhizobial strain specificities, salt and drought resistance, and on inoculation needs and applicable technologies for semi-arid cropland such as those in the western edge of the Loess Plateau.

Any avenue to strengthen the production of legumes in the western Loess Plateau will be worthwhile. The generally inefficient indigenous rhizobia (some of which may be parasitic) and the failure to capitalize on the potential yield increase can be reversed by widespread adoption of inoculation of cultivar-specific strains of *Rhizobium* and incorporation of peas into the newly emerging farming systems being developed in conjunction with local farmers, the Agriculture Bureau of Gansu Province and ACIAR. There is scope for improvement in agronomic practices and increased efficiency of the nitrogen fixation process itself by better management of the symbiotic relationship between plant and bacteria. Research continues on ways and means by which populations of root-nodule bacteria, indigenous or introduced, can be manipulated ecologically, agronomically, edaphically and genetically to improve legume production, and as a consequence, soil fertility and food production.

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