SUPPRESSION OF SOIL-BORNE ROOT PATHOGENS OF ARID LEGUMES BY Sinorhizobium saheli

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ABSTRACT

The productivity of arid legumes in arid and semi-arid tracks remains virtually stagnant over decades because of their susceptibility to root diseases. The information on interaction of beneficial nitrogen fixing rhizobia with particular reference to arid legumes of the region is limited. Systematic studies on predominant species Sinorhizobium saheli in management of root pathogens in arid legumes were undertaken. In-vitro interactions of root pathogens and S. saheli isolated from arid legumes significantly suppressed the growth of all fungal pathogens in presence of S. saheli. In addition the growth of Rhizobium was stimulated in presence of different root pathogens. A field experiment on integrated disease managementexhibited that the maximum root nodulation with the maximum seed yield of 1325 kg/ha was observed from treatment where seeds were treated with S. saheli. Whereas, the minimum root nodulation was recorded in treatment, where a mixture of isolated fungal root pathogens were co-inoculated with S. Saheli was recorded from cowpea. The minimum seed yield was recorded from treatment wherein the mixture of isolated root pathogens of arid legumes was co-inoculated with S. Saheli due to increased disease pressure. The results of in-vitro and in-vivo efficacy of S. saheli strains suggest that their co-inoculation with PGPR's can not only reduce the use of chemical fertilizers but also can significantly enhance yields by increasing plant growth and suppressing soil borne plant pathogenic fungi.

Keywords: Clusterbean, Cowpea, Moth bean, Horse gram, Root pathogens, Management

INTRODUCTION

Clusterbean, cowpea, moth bean and horse gram are predominantly grown in arid and semi-arid tracks of Indian subcontinents under rainfed conditions (Chillar, 2009). During *Kharif*, high temperature coupled with moisture stress put them into abiotic and biotic stresses. The productivity of arid legumes remains virtually

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stagnant over decades because of their susceptibility to root diseases. Most of these root pathogens are soil and or seed-borne. The fungi belonging to the genera *Rhizoctonia*, *Fusarium* and *Neocosmospora* are the most destructive plant pathogens with a very wide host range causing substantial damages and yield reduction in arid legumes (Patel et al., 1998; Fawole et al., 2006; Gautam et al., 2014).

The association between rhizobia and some members of leguminosae accounts for 80% biologically fixed nitrogen and contributes 25-30% of the world wide protein intake. The ability of rhizobia to inhibit certain soil-borne plant pathogens has enlarged the importance of rhizobia besides their use in nitrogen fixation. The dual role of the species of *Rhizobium* in plant growth promotion and disease control is receiving increasing attention in sustainable agriculture (Mallesh, 2008). The antagonistic and plant growth promotion properties of *R. leguminosarum* b.v. *phaseoli* against root rot caused by *F. solani* f.s. *phaseoli* in bean (Buonassisi et al., 1986), and *R. japonicum* against root rot diseases caused by *F. solani* and *M. phaseolina* in soybean have been demonstrated (Al-Ani et al., 2012).

Essalmani and Lahlou (2003) found that cultural filtrate of *R. leguminosarum* and heat killed bacterial cell protected lentil plants from infection by *F. oxysporum* significantly. *In-vitro* hyphal growth of *F. oxysporum* was suppressed by multiplication of *R. meliloti* in a mixed culture, while in soil, it reduced the severity of root rot of lucerne (Sawada, 1982).

Chakraborty and Chakraborty in 1989 experimented that bacterization of seeds with *R. leguminosarum* was highly effective in reducing severity of *F. solani* root rot of pea under field condition but not much antagonistic effect was observed under *invitro* interaction. *Sinorhizobium* sp. strain NGR 234 formed nodules on 112 plant genera (Ausmeeset al., 2004). Singh et al. (2011) found that *S. saheli* isolate (PC-6) from *Prosopis cineraria* was crossed infective to *Acacia senegal*.

The information on interaction of beneficial nitrogen fixing rhizobia with particular reference to arid legumes of the region is limited. Therefore, systematic studies on predominant species *Sinorhizobium saheli* in management of root pathogens in arid legumes were undertaken.

MATERIAL AND METHODS

Field surveys were conducted during Kharif 2012 and 2013 to collect major root pathogens and rhizobia from Jodhpur, Bikaner and Jaipur districts of Rajasthan. Molecular characterized cultures of root pathogens and putative strains of *Sinorhizobium saheli* were used for in-vitroand field evaluation trials (Table-1).

In-vitro interaction

Pure cultures of predominant rhizobial species *Sinorhizobium saheli* were raised in Petri plates containing yeast mannitol agar medium (31.82 g litre⁻¹ containing yeast extract: 1 g, mannitol: 10 g, di-potassium phosphate: 0.50 g,

magnesium sulphate: 0.20 g, sodium chloride: 0.10 g, Congo red: 0.025 g and agar: 20 g in one litre distilled water). Whereas, all fungal pathogenic isolates were raised in Petri plates containing PDA (39 g l⁻¹ containing potatoes infusion form: 200 g, dextrose: 20 g and agar 15 g in one litre dH₂O). Since there are large number of natural and synthetic culture media well known for growing pure cultures of microorganism including rhizobia and fungi, several nutrient media namely PDA, malt extract agar (MEA), nutrient agar (NA), luria broth (LA), yeast mannitol agar (YEMA), rose bengal agar (RBA) and potato rose bengal agar (PRBA) were initially tested for dual cultures for simultaneous luxuriant growth of root pathogens and gram-negative strains of rhizobia for interaction studies.

Since no single tested culture medium supported the luxuriant growth of both fungal pathogens and rhizobia concurrently, a combination of PDA and YEMA in half strength (19.5 g of PDA and 15.9 g of YEMA of Hi Media Company) was used to grow dual cultures. A 5 mm disc of different fungal root pathogens was aseptically transferred onto one side of the Petri plate and a loop full of rhizobial culture was streaked on the other side of the Petri plate and kept for 96 hours in a BOD incubator at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Fungal root pathogens or rhizobial cultures alone on one side of the Petri plate served as control. The relative growth of each microorganism was measured after 96 hours in control (in absence of other microorganism) and in presence of rhizobia for each interaction in four repeats.

Integrated disease management

Field experimentation

A field experiment was conducted in Central Research Farm of Central Arid Zone Research Institute (CAZRI), Jodhpur in randomized block design (RBD) during July, 2013 under rainfed conditions. A putative variety of each arid legume namely cowpea (PGCP- 6), moth bean (CAZRI- Moth 2), guar (RGC- 936) and horse gram (Maru) was treated with rhizobia and or pathogens and then sown in three meter rows in three repeats. The row to row and plant to plant distances were 30 cm and 10 cm, respectively.

Seed pelleting

A sticky solution was prepared by dissolving 100 g of Jaggery and 50 g of Acacia senegal gum in one litre of sterile distilled water by continuous stirring and boiling the water for 15 min. and subsequently cooling it to ambient temperatures. Five ml of sticky solution was mixed with 5 ml of liquid microbial culture with 20 gm of $CaCO_3$ and 60-80 g of seed per replication and allowed to dry for half an hour.

Microbial inoculum

Composite inocula of two *Sinorhizobium saheli* strains was prepared in 200 ml Luria broth culture medium on rotary shaker overnight. Each fungus was multiplied on boiled and sterilized wheat grains mixed with 2% CaSO₄ and 0.5% CaCO₃ for 2 weeks. Pure culture of each fungus was then mixed with 200 ml of sterilized distilled

water and blended in a mixture thrice for 45 seconds. Fungal mycelium and or spores were filtered through doubled layered muslin cloth. The inocula prepared this way were used for seed pelleting as per the treatment required and pathogenicity tests.

Treatments

Each arid legume had nine seed treatments: T_1 = seed treatment with Rhizobia (*Sinorhizobium saheli*), T_2 = mixture of *Macrophomina phaseolina* and *Rhizoctonia bataticola*, T_3 = mixture of *Neocosmospora vasinfecta* and *N. striata*, T_4 = mixture of *Fusarium spp*. (*F. acutatum* + *F. oxysporum* + *F. solani*), T_5 = T_1 + T_2 , T_6 = T_1 + T_3 , T_7 = T_1 + T_4 , T_8 = mixture of all fungal root pathogens and *S. Saheli* and T_9 = control (without any inoculation).

Observations

The effect of seed treatments on root nodulation in different arid legumes was observed at flowering stage. Three plants were uprooted from each replication and a total of nine plants per treatment were taken into account for counting of number of root nodules. Healthy plants were watered before uprooting the plants to keep the root system intact. The uprooted plants were washed in running tap water and then total number of nodules was counted per plant. Whereas, the seed yield was recorded after harvest from each replication comprising of three four meter rows separately and average values of each replication was taken into consideration for assessing the effect of interaction of rhizobia with root rot pathogens on seed yield of arid legumes. The seed yields were converted into killogram (kg) for assessing the effect of root nodulation on root rot pathogens of seed yield.

RESULTS AND DISCUSSION

Isolations from diseased roots of arid legumes revealed association of fungal pathogens belonging to different genera *Macrophomina*, *Fusarium*, *Neocosmospora* and *Syncephalastrum* and healthy roots yielded a predominant rhizobial species *Sinorhizobium saheli* and their identity validated at molecular level (Table 1). No single media tested could support simultaneous luxuriant growth of both types of microorganisms. Finally a combination of two nutrient culture media i.e., PDA (for pathogens) and YEMA (for rhizobia) in half strength supported the growth of both i.e., root pathogens and *S. saheli* equally well. A large number of plants were associated with nodules because it is inherent characteristics of leguminous plants to form nodules under natural growing conditions (Wei et al., 2002; Garcia-Fraile et al., 2007). Earlier researchers also did not find host specificity at species or genus level in different rhizobial species and their leguminous host (Ausmees et al., 2004; Singh et al., 2011).

The data regarding the relative growth of each microorganism measured after 96 hours in absence and presence of rhizobia are presented vide table 2. The combination of PDA and YEMA supported the growth of both fungal pathogens and

rhizobia because the most of the rhizobia are readily isolated on YEMA and fungal pathogens on PDA. *In-vitro* interactions of root pathogens and *S. saheli* isolated from arid legumes significantly suppressed the growth of all fungal pathogens tested in presence of rhizobia. Additionally, the growth of *Rhizobium* was stimulated in presence of different root pathogens. It is evident from the data that *S. saheli* readily grew and showed 42.5 mm growth in absence of root pathogens whereas different fungal root pathogens varied significantly in their rate of growth.

Out of eight fungal strain, three fungal strains namely of *M. phaseolina*, *R. bataticola* and *N. vasinfecta* completed the maximum growth of 85 mm by the end of 96 hrs. Whereas, other five strains namely *N. striata*, *F. acutatum*, *F. oxysporum*, *F. sola*ni and *S. monosporum* showed almost half of the growth which ranged from 42 to 44.5 mm in the stipulated time.

Inhibitory effects on growth of pathogenic fungi by *in-vitro Rhizobium* strains isolated from various legumes have been reported by a number of researchers. Sharif et al. (2003) used dual culture inoculation technique on potato dextrose culture medium. Kucuk (2013) studied interactions between pathogenic fungi and rhizobial strains on yeast glucose mineral agar (YGMA) culture medium by recording inhibition of radial fungal mycelium growth in presence or absence of rhizobia to determine the inhibitory effects of rhizobia against *F. oxysporum*, *F. moniliforme*, *F. solani*, and *F. culmorum* solid media in Petri plates by dividing them in two halves by marking with permanent marker. Rhizobium species significantly reduced wilt and root rot on common bean and chickpea caused by *Fusarium* species (Akhtar and Siddqui, 2007; Arfaoui et al., 2007).

The maximum root nodulation and seed yield was recorded in treatment T_1 where seeds were treated with *S. saheli*. Whereas, the minimum root nodulation and seed yield was recorded in treatment T_8 where a mixture of all the fungal root pathogens were co-inoculated with *S. Saheli*. Whereas, the minimum nodulations in all the arid legumes in treatment T_8 wherein mixture of all the fungal pathogens were co-inoculated with *S. saheli* was due to high competition from root infecting pathogenic fungi and reduced number of nodules as compare to un-inoculated control. The number of nodules did not vary significantly in most of the treatments as compare to control except T_1 because of presence of pathogenic fungi with or without *S. saheli*.

Several species of *Rhizobium* and *Bradyrhizobium* have been reported to restrict the growth of *M. phaseolina*. Biocontrol mechanism of rhizobia may involve the production of antibiotics, HCN, siderophores and/or phytoalexins which acts against plant pathogenic microorganism.Inhibition of microbes was most likely due to expression of essential metabolites produced in excess by the interacting

microorganisms in a medium where it is in limited supply (Arfaoui et al., 2007). The amount and variety of nutrients available to the interacting organisms in the soil are very different to those of culture media (Kucuk, 2013).

Treatment T_1 resulted in the maximum seed yield due to inoculation with mixture of S. saheli strains that had no competition with any root pathogenic fungi. The reduction in seed yield in other treatments as compare to control was due to attack by root rot pathogenic fungi. The treatment T_4 followed by treatment T_2 wherein mixture of Fusarium spp.and M. phaseolina + R. bataticola were coinoculated as seed treatments resulted in the minimum seed yields because of absence of growth promoting S. saheli strains. Co-inoculations of S. saheli with one of the major pathogenic fungi i.e. treatment T_5 (S. saheli + M. phaseolina), T_6 (S. saheli + Neocosmospora spp.) and T_7 (S. saheli + Fusarium spp.) resulted in better yields than in treatments T_2 , T_3 and T_4 where seeds were treated with only one major pathogenic fungus. This clearly indicates the inhibitory effect of S. saheli on different pathogenic fungi in all the four arid legumes tested.

Species of *Rhizobium* have been shown to reduce root rotsand wilts in legumescaused by *M. phaseolina, R. solani* and *Fusarium spp.* (Haque and Ghaffar, 1993, Akhtar and Siddqui, 2007; Arfaoui et al., 2007). Khalequazzaman and Hossain (2008) suggested that seed treatment with *Rhizobium* strain BINAR P36 not only control foot and root rot but also increased green pod yield in *P. vulgaris*.

Hossain et al. (2000) observed reduction in fusarial foot and root rot in chickpea over untreated control with *Rhizobium* inoculation @ 50 g kg⁻¹ seed. Treatments of seeds with bio-fertilizer substantially reduced death of plant in lentil and chickpea due to infection by *S. rolfsii*. Kumar et al. (2006) assessed the genetic diversity of PGPR isolated from *Macuna pruriens*. Among the five isolates, only MPR4 inhibited the growth of *M. phaseolina* and *F. oxysporum*. They observed that fungal inhibition was more pronounced in dual culture conditions in comparison to that of cell free culture filtrate. Huang and Erickson (2007) proved that seed treatment with *R. leguminosarum* b.v. *viceae* was effective in controlling damping off in pea under naturally infested field condition.

Mazen et al. (2008) evaluated the antagonistic activities of the cultural filtrates of *R. leguminosarum* and arbuscular mycorrhiza (AM) fungi in the management of damping off and root rot diseases of faba bean. They observed that soaking of faba bean seeds in cultural filtrate of *Rhizobium* species individually or in combination with AM fungi not only act as bio-protection agents against root rot diseases but also substantially increase plant growth and yield.

Effect of seed treatment on root nodulation in arid legumes is presented in figure 1. Nine seed treatments resulted in significant variations in number of root nodules and seed yield. The data clearly exhibited that the maximum root nodulation as observed in treatment T₁ where seeds were treated with *S. saheli* strain CP-R4.

Whereas, the minimum root nodulation was recorded in treatment T_8 where a mixture of all the fungal root pathogens were co-inoculated with *S. Saheli*. Nevertheless, statistically most of the treatments except T_1 were at par with that of control.

Effect of seed treatment on seed yield in arid legumes is shown in figure 2. The maximum seed yield of 1325 kg ha^{-1} was recorded from cowpea and the minimum seed yield of 483 kg ha^{-1} was recorded from horse gram. The treatment T_1 resulted the maximum seed yield which was significantly higher compared to all other treatments. The minimum seed yield was recorded from treatment T_8 where the mixture of all the root pathogens of arid legumes was co-inoculated with *S. saheli* due to increased disease pressure.

Significantly higher number of nodules was recorded from treatment T₁ where mixture *S. saheli* strains were used for seed coatings was because *S. saheli* has a wide host range and therefore could infect all the four arid legumes tested (Rasanen et al., 2001; Singh et al., 2011). *Sinorhizobium* species strain NGR 234 formed nodules on more than 100 plant genera (Ausmees et al., 2004). Singh et al. (2011) found that *S. saheli* isolate (PC-6) from *Prosopis cineraria* was cross infective to *Acacia senegal*. Chickpea rhizobia were both phenotypically and genotypically diverse and were closer to *Mesorhizobium* or *Sinorhizobium* species and distinct from *Bradyrhizobium* (Maatallah et al., 2002). *R. leguminosarum* biovars confer host specificity on nodulation such as biovar *viciae* on peas, biovar *phaseoli* on beans etc. Whereas, symbiotic genes are carried on plasmids which are interchanged (Young et al., 2006).

Seed dressing or soil drenching with *R. leguminosarum* reduced the infection of *M. phaseolina*, *R. solani* and *Fusarium* spp. in both leguminous and non-leguminous plants under field conditions (Haque and Ghaffar, 1993). Huang and Erickson (2007) observed significantly reduced incidence of damping-off, promoted seedling growth and increased root nodule mass, plant biomass and seed yield of pea and lentil due to Elkoca et al. (2008) observed that seed inoculation of chickpea with *Rhizobium* significantly increased the seed yield under different inoculation treatments which ranged between by 18.0% to 30.5 % over the control.

CONCLUSION

Drawbacks of intensive farming practices and environmental costs of nitrogen (N) and phosphorus fertilizers have renewed interest in biofertilizers. The results of *in-vitro* and *in-vivo* efficacy of *S. saheli* strains suggest that their co-inoculation with PGPR's can not only reduce the use of chemical fertilizers but also can significantly enhance yields by increasing plant growth and suppressing soil borne plant pathogenic fungi.

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Table 1. Molecular Identification of fungal root pathogens and rhizobial strains of arid legumes

Origin	Isolate	Molecular Identification	Gen Bank accession number
Jodhpur	JD-HG1	Macrophomina phaseolina	JQ954868
Jodhpur	JD-CP5	Rhizoctonia bataticola	JQ954872
Jodhpur	JD-HG10	Neocosmospora vasinfecta	JQ954881
Jaipur	JAI-CP11	Neocosmospora striata	JQ954882
Jaipur	JAI-CP16	Fusarium acutatum	JQ954883
Bikaner	BK-CB15	Syncephalastrum monosporum	JQ954886
Bikaner	BK-CB20	Fusarium solani	JQ954888
Jaipur	JAI-CB37	Fusarium sp.	JQ954890
Jaipur	JAI-MB24	Fusarium oxysporum	JQ954892
Jodhpur	HG-R4	Sinorhizobium saheli	KC794787
Jodhpur	HG-R6	Sinorhizobium saheli	KC794788

Table 2. *In-vitro* interactions of root pathogens and *Rhizobium* (*S. saheli*) isolated from arid legumes

Isolate	Pathogen	Growth (mm)		
		Fungus alone	Fungus + Rhizobium	Rhizobium alone
JD-HG5	M. phaseolina	85	40 + 45	42.5
BK-MB8	R. bataticola	85	42 + 43	42.5
BK-CB17	N. vasinfecta	85	39 + 46	42.5
JAI-CP11	N. striata	42	38 + 46	42.5
JAI-CP16	F. acutatum	44.5	36 + 47	42.5
JAI-MB24	F. oxysporum	43.5	38 + 43	42.5
JAI-MB21	F. solani	42	35 + 48	42.5
BK-CB15	S. monosporum	44	41 + 44	42.5
CD at 0.05%		3.3	1.2; 2.1	0.8

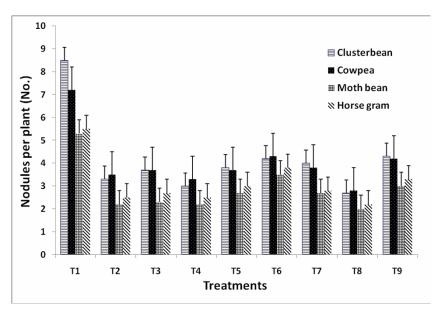


Figure 1. Effect of seed treatment on root nodulation in arid legumes

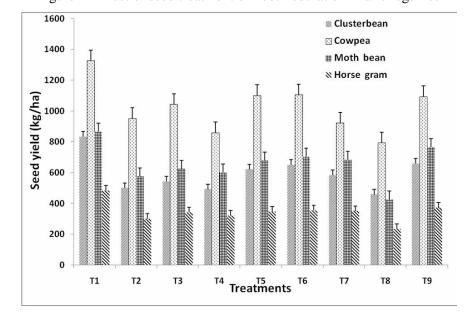


Figure 2. Effect of seed treatment in seed yield in arid legumes