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Comparative plant growth promoting potential of psychrotolerant diazotrophs, *Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 against native *Cajanus cajan* (L.) and *Eleusine coracana* (L.)

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Two Himalayan diazotrophs, *Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 were examined for their plant growth promoting properties against the native crops, *Cajanus cajan* and *Eleusine coracana*. *Pseudomonas* sp. JJS2 was found to be more effective for *C. cajan*, whereas *Enterobacter* sp. AAB8 showed better plant growth promotion in *E. coracana.* The inoculation of JJS2 significantly ($p<0.05$) increased the fresh weight (93.73%), dry weight (52.58%) root length (38.02%) and shoot length (38.03%) in the legume *C. cajan*; while, in the case of *E. corocana*, the increment was lesser but still significant. Similar results were obtained with AAB8 where it showed better plant growth promotion in *E. coracana* plants by stimulating the fresh weight (7.0%), dry weight (58.42%), shoot length (8.52%) and root length (28.06%). In the case of *C. cajan*, the effect of AAB8 strain was also significant as shown by the increment in the plant growth parameters. Biochemical parameters in each treatment *viz.* nitrate reductase activity and chlorophyll content were also found to be enhanced significantly. The results suggest that *Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 strain could be used effectively as a low cost bioinoculant in high altitude agricultural belts.

Key words: Psychrotolerant, bioinoculant, microflora, diazotrophs.

INTRODUCTION

Agriculture plays an important role in sustaining livelihood of local people in marginal land of Himalayan ecosystems. These regions are characterised by extreme climatic conditions as winter temperature plunges below freezing, wide diurnal fluctuations in temperature, scanty rainfall, low air pressure and strong solar radiation. Agriculture is therefore said to be a difficult task in such conditions where production of crops suffer from various drawbacks; one of which is nitrogen (N) deficiency. Being an organic state, use of chemical fertilizers for crop production is not recommended.

Hence, the application of bioinoculant composed of

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diazotrophic bacteria as an alternative to nitrogen fertilizers has emerged as a promising approach.

Eleusine coracana (Finger millet) is grown in the Western Indian Himalaya (WIH) up to an elevation of 2,300 m and is one of the major components of the local cuisin. Finger millet is especially valuable as it contains the amino acid methionine and other nutritious elements (Gopalan et al., 2002). It is grown as a mixed crop with pulses whose production is very poor in upper Himalayas. *Cajanus cajan* (pigeonpea) is a nutritious legume crop with high protein and mineral content (Cook et al., 2005). It is generally a warm-season, deep-rooted plant whose specific hardiness and day-length requirements vary by cultivar, though most require 90 to 120 frost-free days annually (Cook et al., 2005). These conditions are very hard to achieve in the high altitude agriculture lands and hence, their production in the Himalayan hilly regions is almost negligible. Therefore, there is great interest in agriculture for microbial bio-inoculants that enhance crop production under cold conditions. Significance of rhizosphere microbiome under several biotic and abiotic stresses has been reviewed by Mendes et al. (2013). Previously presence of psychrotolerant diazotrophs of Western Indian Himalayas was reported by Suyal et al. (2014a). We, therefore, conducted the present study which focuses on the isolation, identification and application of native psychrotolerant diazotrophic strains under natural fluctuating temperatures which could be explored against the individual or mixed cropping of native *E. coracana* and *C. cajan* in upper Himalayan agricultural lands.

MATERIALS AND METHODS

Bacterial isolates and screening of isolates for N_2 fixation

In total, 31 bacterial isolates were isolated from soil samples from Western Indian Himalayas. All were screened for diazotrophy by their *nif*H gene amplification (Poly et al., 2001) and then their growth on nitrogen-free medium (Burk's medium, Himedia). The selected isolates were identified at the National Centre of Cell Science, Pune University Campus, Pune, using 16S rDNA sequencing (Antony et al., 2010).

Plant growth promotion studies

The pot trial was performed at Pantnagar (244 m, 28.97°N, 79.41°E), a *Tarai* region of Indian Shiwalik Himalayas, during the month of October to November. In brief, the soil was loamy in texture and soil pH was 7.2. Pots were kept in a net house having natural fluctuating temperature ranging from 30±5°C in day to 10±5°C in night, for 60 days (Katiyar and Goel, 2003; Rani et al., 2013). Psychrotolerant diazotrophs, *Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 were cultivated aerobically in Burk medium at 28°C. The seeds of the crops were bacterized (10^8 cells/seed) using carboxymethyl cellulose with both the strains separately (Katiyar and Goel, 2003). Non-bacterized seeds served as control. Three replicates for each treatment were taken. Agronomical parameters (fresh weight, dry weight, root length and shoot length) of plants were measured after 50 days of germination.

Nitrate reductase activity and chlorophyll assay

The nitrate reductase activity of plant flag leaves was measured according to Hageman and Hicklesley (1971). The total chlorophyll content of plant flag leaves was measured according to Rani et al. (2009).

Statistical analysis

The pot experiment was performed with three replicates per treatment. Data were analyzed by ANOVA using statistical software STPR 2 in Department of Mathematics, Statistics & Computer Science GBPUA&T Pantnagar. Mean difference of the treatments was considered to be significant at the 5% level.

RESULTS AND DISCUSSION

Screening of the diazotrophs

Among 31 isolates, only 2 *viz.* JJS2 and AAB8 showed good growth on N_2 free media at room temperature and subsequently gave an amplification of 360 bp *nif*H fragment confirming their diazotrophic trait. On the basis of the presence of diazotrophic trait, two psychrotolerant bacterial isolates JJS2 and AAB8 were selected for further study and identified using 16S rDNA sequencing technique as *Pseudomonas* sp. (Accession no. HQ222365) and *Enterobacter* sp. (Accession no. HQ222364), respectively. The psychrotolerant characteristic of the isolates is supported by the fact that the isolation site of these isolates: Almora and Jageshwar (Table 1) was having sub tropical climate where temperature varies from 30°C to near freezing and both selected strain (JJS2 and AAB8) were able to grow on nitrogen deficient medium at 10°C in low temperature incubator.

Previously, it was confirmed that Western Indian Himalayan soil has bacterial community with a tremendous potential of biodegradation (Soni et al., 2008) and plant growth promotion properties (Suyal et al., 2014a, b; Rani et al., 2013; Singh et al., 2012). Premalatha et al. (2009) also highlighted the prevalence of many important genes/proteins from icy heights of the Himalayas. Recently, seven cold adapted bacterial diazotrophs from the Red Kidney bean (*Phaseolus vulgaris* L.) rhizosphere of WIH were isolated and proteome of the psychrophilic nitrogen fixing *Pseudomonas migulae* strain S10724 for low temperature diazotrophy was documented (Suyal et al., 2014c).

Evaluation for plant growth promotion under nethouse conditions

The productivity of pulses is very poor in upper Himalayas. It has been postulated that the rhizospheric microorganisms contributes to the ability of some plant species to survive under extreme conditions (Jorquera et al., 2012). Nevertheless, it is interesting to note that despite

Table 1. Characterization of the N_2 fixing bacterial strains used in the present study.

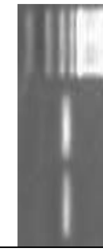
S/N	Bacterial strains	Isolation Site	Climate	Longitude/ latitude	Elevation (m)	Cell morphology	Grams reaction	*nifH* amplification
1	*Pseudomonas* sp. JJS2 (HQ222365)	Jageshwar	Subtropical	29.39°N, 79.34°E	1646	Very short thin rod	-ve	
2	*Enterobacter* sp. AAB8 (HQ222364)	Almora	Subtropical	29.62°N, 79.67°E	1651	Thick small rod	-ve	

Table 2. Two-way ANOVA depicting the effect of *Pseudomonas* sp. JJS2 and *Enterobacter* Sp. AAB8 on *C. cajan* and *E. coracana* under greenhouse conditions after 50 days of germination, respectively.

Species	Treatment	Nitrate reductase activity (mmol NO_2 g^{-1} fr. wt)[a]	Chlorophyll content (mg g^{-1} fr. wt)[a]	Fresh weight (g)[a]	Dry weight (g)[a]	Shoot length (cm)[a]	Root length (cm)[a]
Cajanus cajan	Control	0.817	1.83	3.35	2.32	36.23	16.2
	JJS2	1.81 (121.54)	3.21 (75.41)	6.49 (93.73)	3.54 (52.58)	50.01 (38.03)	22.36 (38.02)
	AAB8	0.846 (3.5)	2.73 (49.18)	4.66 (39.1)	2.83 (21.98)	36.53 (0.83)	19.00 (17.28)
Eleusine coracana	Control	0.119	1.302	0.899	0.368	18.42	19.60
	JJS2	0.306 (157.14)	1.46 (11.82)	0.90 (0.33)	0.45 (23.09)	18.66 (1.30)	20.30 (3.57)
	AAB8	0.408 (54.62)	1.53 (17.51)	0.96 (7.00)	0.58 (58.42)	19.99 (8.52)	25.10 (28.06)
SEM ±		0.36	0.79	0.14	0.53	0.89	0.59
Critical difference at 5%		0.11	0.24	0.43	0.16	2.75	1.21

a: Each value is the mean of three replicates; Value in the parentheses indicates percentage increase with respect to control.

the impact of low temperatures on nodule formation and nitrogen fixation, native legumes in the high arctic can nodulate and fix nitrogen at rates comparable to those reported for legumes in temperate climates (Bordeleau and Prevost, 1994).

Data obtained from the pot trial in respect of agronomical and biochemical parameters of both the crops with different treatments are presented in Table 2. Differences between the inoculated treatments were evident when compared with non-inoculated control plants (Figures 1 and 2). The effect of *Pseudomonas* sp. JJS2 strain on *C. cajan* and *E. coracana* was significantly higher with an increase in root length of 38.02 and 3.57%; shoot length of 38.03 and 1.30%; fresh weight of 93.73 and 0.33%; and dry weight of 52.58 and 23.09%, respectively, over control (Table 2). The total chlorophyll content was also higher in *C. cajan* and *E. coracana* plants inoculated with strain JJS2 with an increase of 75.41 and 11.82%, respectively. Increased chlorophyll content and hence increased photosynthesis enhanced plant growth and yield (Rani et al., 2013, Singh et al., 2012). Significant increase in nitrate reductase activity in inoculated *C. cajan* and *E. coracana* plants was observed by 121.54 and 157.14%, respectively (Table 2). The reduced nitrogen input to the plant is determined by the activity of nitrate reductase, which catalyses the first step and determines the rate of this assimilating process that acts as a limiting factor of plant growth and development

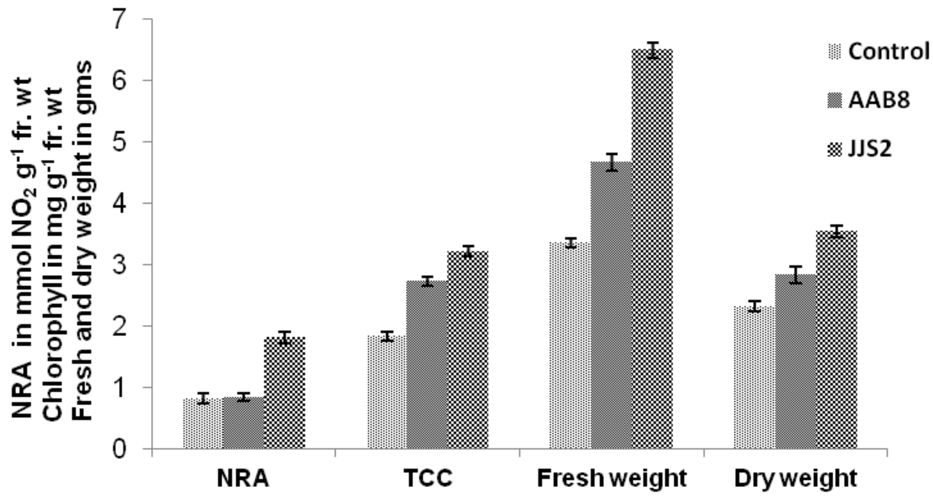


Figure 1. Effect of *Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 on nitrate reductase activity (NRA), total chlorophyll content (TCC), fresh weight and dry weight of *Cajanus cajan* after 50 days of germination.

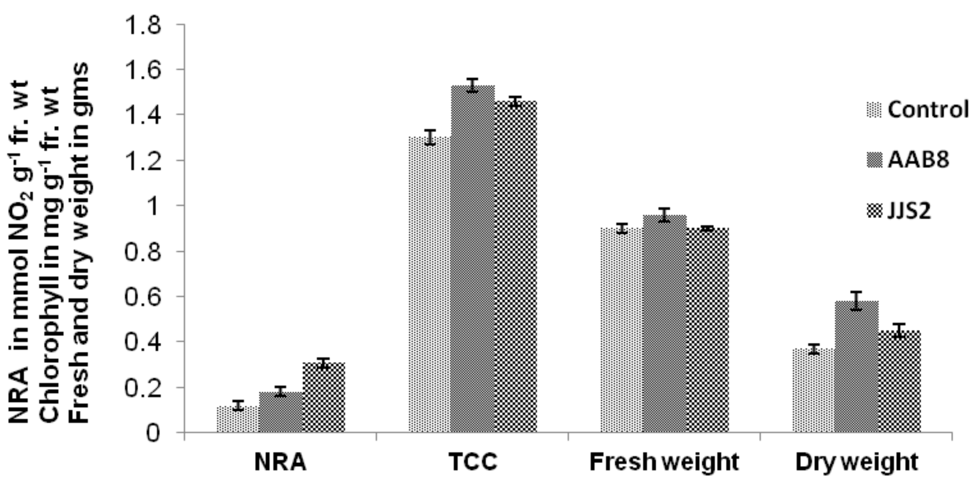


Figure 2. Effect of *Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 on nitrate reductase activity (NRA), total chlorophyll content (TCC), fresh weight and dry weight of *Eleusine coracana* after 50 days of germination.

(Solomonson and Barber, 1990). Similarly, inoculation of *Enterobacter* sp. AAB8 in *C. cajan* and *E. coracana* plants increased all the agronomical parameters significantly *viz.* root length by 17.28 and 28.06%; shoot length by 0.83 and 8.52%; fresh weight by 39.1 and 7%; dry weight by 21.98 and 58.42%, respectively (Table 2). Chlorophyll content by 49.18 and 17.51% and nitrate reductase activity by 3.5 and 54.62% was also found to increase in AAB8 strain inoculated *C. cajan* and *E. coracana* plants (Table 2).

In terms of efficiency, *Pseudomonas* sp. JJS2 strain was found to be more efficient bioinoculant for *C. cajan* (legume) than *E. coracana* (cereal) (Figure 1). Contrary to this, the effect of *Enterobacter* sp. AAB8 strain was more pronounced in a cereal than legume (Figure 2). Conclusively, both the psychrotolerant strains promote the growth of native *C. cajan* and *E. coracana* plants which indicates their utility as a successful bioinoculant for individual or mixed cropping at high altitude agricultural systems. But inoculation of soil with such beneficial microorganisms may not result in significant effects unless the environment supports growth and survival of the introduced microorganisms. In this context, the relevance of locally adapted strains of bio-inoculants gains paramount importance since most often the introduced strains fail to produce the desired effects of inoculation.

Plant growth promotion by bio-inoculation with PGPR has been frequently reported. Katiyar and Goel (2003) assessed the inoculation effect of phosphate-solubilizing cold-tolerant mutant of *P. fluorescens* on mungbean in sterilized and unsterilized soil and observed that inoculated plants resulted in better plant growth in both soils. Moreover, it is also reported that inoculation of the diazotrophic bacterial strains significantly increased the biomass of plants and also had a significant impact on the total N in plant tissues as compared to uninoculated control (Suyal et al., 2014a, Yim et al., 2009). These reports further corroborates with our findings with respect to efficacy of a native diazotrophic strain in improving crop growth parameters and hence increasing crop yield.

Our findings provide evidence that these two bioinoculants *viz. Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 can withstand extremities of cold and retain their functional traits even at unfavourable conditions. It can thus be concluded from the present study that *Pseudomonas.* sp. JJS2 and *Enterobacter* sp. AAB8 play an important role in plant growth promotion of native Himalayan crops and can be further exploited for growth and yield enhancement in mountain agricultural systems.

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Conflict of interests

The author(s) did not declare any conflict of interest.

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