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Potassium and *Rhizobium* application to improve quantitative and qualitative traits of lentil (*Lens culinaris* Medik.)

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Abstract

In an effort to improve plant growth and productivity by increasing the amount of N_2-fixation, a two-year field experiment was designed to study the combined effect of potassium and two strains of *Rhizobium leguminosarum* (L-1897 and L-2097) on quantitative and qualitative traits of lentil (*Lens culinaris* Medikus). Bacterial inoculation and potassium application (0 kg K ha^{-1} and 50 kg K ha^{-1}) caused measurable changes in the observed characteristics in both years of study. In addition to growth characteristics, chemical- and bio-fertilizer treatments also affected the nitrogen and potassium concentration in seeds and seed protein content. Yield and yield characteristics improved more with the combined application as compared with a single treatment and control. Among the bacterial strains, L-1897 along with potassium fertilization resulted in highest yield. We conclude that optimum potassium fertilization is required for the favorable and sustained action of *Rhizobium* to influence growth characteristics and qualitative traits and hence, yield and yield components.

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Introduction

At present India produces about 259 million tonnes or little more of food grains every year, which is barely sufficient to feed the current population of over 1 billion people. The population of the country is expected to grow at about 1.34% per annum and it is expected to overtake China by the year 2030. Estimated food requirement will be more than 300 million tonnes by 2025 and about 450 million tonnes by 2050 (Suryapani, 2009). In India, there have been a number of reports from almost every state in the country that in spite of use of increased inputs, crop production has either levelled off or declined (see Römheld and Kirkby, 2010). The factors that have created the decline in crop production are multiple and complex, including higher global prices for energy. One of the solutions is to increase the application of fertilizers in an efficient and environmentally sensitive manner so that food production may be increased without polluting the environment. Currently, we are using about 23.5 million tonnes of nitrogen (N), phosphorus (P) and potassium (K) nutrients per year. However, fertilizer factor productivity has declined across years. From 15 kg grain/kg nutrients in the 1970's, it has come down to merely 6 kg grain/kg nutrients (Suryapani, 2009). Considering these facts, there is now growing interest in biological N fixation all over the world, as it is considered an important phenomenon for environment-friendly and sustainable food production.

Legumes have always been used by mankind as a protein rich diet and for soil improvement. Legumes can symbiotically fix atmospheric N through *rhizobia* and therefore need minimal fertilizer N applications. Potassium is considered necessary for proper development and functional longevity of root nodules as it helps in the translocation of sufficient amount of photosynthates to root nodules. This is of great importance, particularly towards the physiological maturity of the plant as there is a reduction in N fixation and hence leghemoglobin and protein content of root nodules. Therefore, for optimum symbiotic N_2-fixation and N-partitioning towards reproductive parts and nodules, K plays an important role as it affects some parameters like size, weight and number of nodules (Premaratne and Oertli, 1994). In our earlier report, K application was found to reduce the requirement of fertilizer N in a wheat/lentil intercropping system (Suryapani *et al.*, 2013).

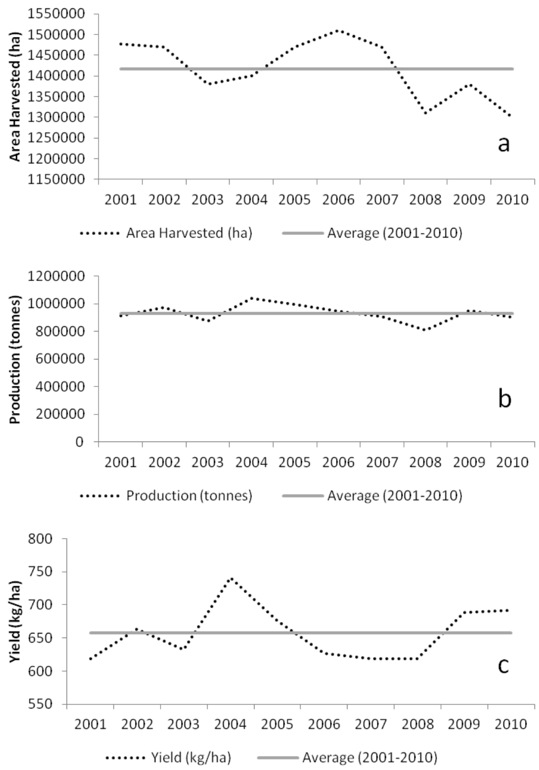

Fig. 1. Area harvested (a), production (b) and yield (c) of lentil in India in the last decade (2001-2010). Source: FAOSTAT, 2012.

An annual, semi-erect and short legume, lentil (*Lens culinaris* Medikus) is among the most ancient domesticated crops. Its seeds have nutritional value, as they are high in protein. As in the case of other annual grain legumes, lentil has the ability to maintain soil fertility by fixing atmospheric N in symbiosis with *rhizobia* present in root nodules. Indian subcontinent is the leading lentil producing area in the world (see Thavarajah *et al.*, 2011). In India, there was a decline of 1.36% in the yield of lentil during 2001-2010 (average yield was 657.72 kg ha^{-1}) compared with 1991-2000 (average yield was 666.67 kg ha^{-1}). A yield decrease of 2.71% was observed during 2006-10 (average yield was 648.92 kg ha^{-1}) as compared with 2001-05 (average yield was

666.52 kg ha^{-1}) (FAOSTAT, 2012) (Fig. 1). Considering these facts and also the fact that fertilizer factor productivity has been progressively declining, we undertook a two-year field study to evaluate the effect of K on association of two *rhizobia* strains relative to qualitative and quantitative traits and hence yields and yield characteristics of lentil under Delhi (north India) climatic conditions.

Materials and methods

Experimental site

The experiments were carried out at the experimental field of Hamdard University, New Delhi, India during 2007-2009 (two growing seasons, i.e., 2007-08 and 2008-09, designated in the paper as Year 1 and Year 2, respectively). Experimental farm soil was neutral (pH 7.1) with 0.28% (w/w) organic carbon content, 128 kg/ha available N, 14 kg/ha available P, 119 kg/ha available K and had a sandy loam texture.

Treatments and plant material

Two rates of K (0 and 50 kg ha^{-1}, designated in the experiment as K1 and K2, respectively) along with two *Rhizobium leguminosarum* strains (L-1897 and L-2097; designated in the experiment as R1 and R2, respectively) were tested on the lentil variety (L-4076). L-4076 is a bold seeded moderately resistant to rust lentil variety. Having a yield potential of about 1000-1600 kg ha^{-1}, it is recommended for general cultivation in India (see Krishnareddy and Ahlawat, 1996). *Rhizobia* strains and seeds of lentil were obtained from Division of Microbiology and Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi, respectively. *Rhizobia* strains were maintained on yeast extract mannitol at 4ºC. Whenever required, they were multiplied on yeast extract mannitol broth. Before sowing, uniform basal doses of 20 kg ha^{-1} of N (in the form of urea) and P (in the form of single super phosphate) along with K1 or K2 (in the form of muriate of potash - KCI) were applied to all the plots. Prior to sowing, the seeds were treated with two *rhizobia* strains separately. Three hundred seeds were hand-planted in each plot (2 m × 2 m plots) in ten rows with spacing of 20 cm. Thirty seeds were sown in each row. Plants were maintained at a distance of 15 cm with in a row by thinning after germination.

Growth and yield analysis

Sowing was done in complete randomized block design (CRBD) and sampling was carried out by taking randomly selected plants in three interior rows in a plot, excluding the marginal rows. Sampling for plant height, number of nodules, protein content in leaves and nodules, and leghemoglobin (Lb) content was carried out at 30 days after sowing (DAS), 60 DAS and 90 DAS corresponding to the three stages of plant development, i.e., pre-flowering, flowering and post-flowering. Plant height was measured with a meter scale. Plants were uprooted and number of nodules on each root was counted. Bradford (1976) was followed for the extraction of protein content in leaves (usually 3rd leaf from the top of randomly selected plants from a plot) and fresh nodules. The extracted samples were read on UV-VIS spectrophotometer (Lambda Bio 20, Perkin Elmer). Leghemoglobin content was determined by the method of Appleby and Bergersen (1980). Fresh nodules were mixed with 3 volumes of phosphate buffer and macerated. The contents were filtered through two layers of cheesecloth. Nodule debris was discarded and remainder brown filtrate was centrifuged and diluted. To an equal volume of extract, alkaline pyridine reagent was added and mixed. The resulting hemochrome was equally divided into two portions. To one portion, a few crystals of sodium dithionite were added to reduce the hemochrome and stirred without aeration. To the other portion, a few crystals of potassium hexacyanoferrate were added to oxidize the hemochrome and the contents of both the test tubes were measured at 556 nm and 539 nm, respectively. Leghemoglobin content was calculated using the following formula:

$$\text{Lb content (mM)} = [(A_{556} - A_{539})/23.4] \times 2D$$

where D is initial dilution.

For elemental analysis, seeds were properly washed, dried, finely powdered, sieved through a 72 mm mesh screen, stored in polyethylene zip pouches, labelled and used for analysis later. Lindner (1944) was followed for the estimation of K in the digested samples using flame photometer, pre-adjusted at 0, 25, 50 and 100 ppm readings. The readings were recorded directly in ppm and converted to actual value of K in seed samples by multiplying with a dilution factor. It was expressed as % DW. Nitrogen percentage was analyzed by packing the known weight of seed sample-powder in tin boats with the help of Elementar system (CHNS Analyzer, Vario EL.) and was expressed as % DW. Nitrogen content in seed was multiplied by 6.25 to get the total seed protein content as per Bremner (1996). For yield and yield component analysis, number of pods plant^{-1}, 1000-seed weight (g plant^{-1}) and grain yield (kg ha^{-1}) were recorded for each replicate at harvest. Spikes were shelled by hand and then weighed.

Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) using SPSS (Statistical Procedure for Social Sciences, v. 11.0, Chicago, IL). The least significant difference (LSD) was calculated for $P < 0.05$.

Results

As evident from Table 1, the difference among treatments is significant in all parameters except protein content of leaves, seed N content and seed protein content where as the year's difference is non-significant in all parameters except leghemoglobin content of nodules, number of pods and 1000 seed weight.

Table 1. Summary of analysis of variance and mean squares for various parameters measured in 2008 and 2009 for lentil.

Source of variation	df	Mean square: Plant height (cm)			No. of nodules (plant^{-1})			Protein content of leaves (mg g^{-1} FW)			Protein content of nodules (mg g^{-1} FW)		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Y	1	9.152	49.665	0.097	0.833	13.333	19.200	2.864	11.384	11.139	3.287	2.220	0.954
T	4	77.080*	136.295*	158.002*	37.550*	221.617*	118.383*	3.728*	4.742	4.566	10.875*	10.281*	21.748*
Y × T	4	0.319	1.731	3.148	1.417	7.250	5.950	0.002	0.026	0.050	0.014	0.070	0.003
Error	20	7.501	11.203	5.904	5.233	19.833	20.933	1.039	4.750	4.591	1.348	0.984	0.381

Source of variation	df	Leghemoglobin content of nodules (mM)			Seed K content (%)	Seed N content (%)	Seed protein content (%)	Number of pods (plant^{-1})	1000 seed weight (g)	Seed yield (g plant^{-1})	Seed yield (kg ha^{-1})
		30 DAS	60 DAS	90 DAS							
Y	1	0.001065*	0.000236*	0.000023	0.236	0.329	12.871	2100.033*	18.881*	0.173	8909.633
T	4	0.000121*	0.000748*	0.000313*	1.106*	0.102	3.994	829.617*	21.063*	1.490*	156496.950*
Y × T	4	0.000002	0.000003	0.000001	0.0276	0.003	0.106	49.950	2.035*	0.005	413.55
Error	20	0.000007	0.000049	0.000023	0.1236	0.138	5.398	25.533	0.270	0.049	8140.667

*significant at $P < 0.05$; DAS (days after sowing); Y (year); T (treatment)

Plant height and number of nodules

Plant height of lentil significantly increased in both the growing seasons under *Rhizobium* inoculation and K application. The effect of R_2 and K_{50} was more pronounced at the three stages as compared with R_1 and K_{50}. Plant height ranged between 13.45 cm at 30 DAS (R_0K_0, Year 1) to 45.85 cm at 90 DAS (R_2K_{50}, Year 2) (Table 2). As evident from Table 2, number of nodules increased with K application and *Rhizobium* inoculation in both years of study. The lowest number of nodules (5.00 nodules plant^{-1}) and highest number of nodules (25.33 nodules plant^{-1}) in this experiment was recorded in R_0K_0 (30 DAS, Year 1) and R_2K_{50} (90 DAS, Year 1), respectively. R_2K_{50} treatment increased the number of nodules at 60 DAS by 204.17% and 114.71% in Year 1 and Year 2, respectively, over the control.

Protein content of leaves and nodules

Application of *Rhizobium* and K enhanced the protein content in both Year 1 and Year 2 significantly over R_0K_0 treatment. Higher protein content in leaves was recorded at 60 DAS, compared with the other two stages. In general, *Rhizobium* inoculation increased protein content, but along with K application, it enhanced the total soluble protein to 12.21 and 13.54 mg g^{-1} FW at 60 DAS in Year 1 and Year 2, respectively. R_2K_{50} had the maximum protein content, which was significantly higher than that from R_1K_{50} treatment. R_0K_0 had the lowest value for protein content in both the growing seasons at all three stages of plant growth (Table 3). Protein content of nodules was also enhanced by K application and *Rhizobium* inoculation in both years of study, showing significant differences from the control (R_0K_0). The highest protein content in nodules was recorded with R_2K_{50} treatment in both years and the enhancement was highest at 60 DAS. Nodule protein content increased to 35.54 and 34.27 mg g^{-1} FW at 60 DAS in Year 1 and Year 2, respectively, a significant increase over the control (R_0K_0) (Table 3).

Table 2. Effect of *Rhizobium* inoculation and potassium application on plant height and number of nodules of lentil.

Stage /Year	Treatments						LSD at 5%		
	R_0K_0	R_1K_0	R_1K_{50}	R_2K_0	R_2K_{50}	Mean	Y	T	Y × T
	Plant height (cm)								
30 DAS/Y1	13.45±1.47	16.00±1.31	17.66±0.82	19.23±0.70	23.21±1.63	17.91±1.83			
30 DAS/Y2	14.94±2.81	16.58±2.31	18.83±2.55	20.83±2.49	23.90±1.97	19.01±1.76			
Mean	14.19^{d}±1.05	16.29^{c}±0.41	18.25^{bc}±0.83	20.03^{b}±1.13	23.55^{a}±0.49		NS	2.08	NS
30 DAS/Y1	24.44±1.70	25.46±2.03	28.12±1.93	32.56±2.25	34.98±2.57	29.11±2.27			
30 DAS/Y2	26.28±1.45	28.54±1.52	30.45±1.76	34.02±2.66	39.14±4.36	31.69±2.52			
Mean	25.36^{d}±1.30	27.00^{cd}±2.18	29.29^{c}±1.65	33.29^{b}±1.03	37.06^{a}±2.94		NS	2.69	NS
30 DAS/Y1	31.16±1.50	34.81±1.31	37.27±0.66	40.28±1.70	43.19±1.80	37.34±2.34			
30 DAS/Y2	30.85±2.13	34.63±2.43	36.43±1.87	39.53±1.13	45.85±1.93	37.46±2.82			
Mean	31.01^{e}±0.22	34.72^{d}±0.13	36.85^{c}±0.59	39.91^{b}±0.53	44.52^{a}±1.88		NS	1.95	NS
	No. of nodules (plant^{-1})								
30 DAS/Y1	5.00±1.22	5.00±1.22	7.00±1.87	9.67±1.78	11.33±2.27	7.60±1.42			
30 DAS/Y2	5.33±1.78	7.00±1.41	7.00±0.71	9.33±1.08	11.00±2.12	7.93±1.11			
Mean	5.17^{d}±0.24	6.00^{cd}±1.41	7.00^{c}±0.00	9.50^{b}±0.24	11.17^{a}±0.24		NS	1.75	NS
30 DAS/Y1	8.00±1.87	9.00±1.87	12.33±2.16	18.67±2.86	24.33±4.81	14.47±3.46			
30 DAS/Y2	11.33±3.34	13.00±2.12	12.67±1.47	17.67±2.27	24.33±5.76	15.80±2.67			
Mean	9.67^{c}±2.36	11.00^{c}±2.83	12.50^{c}±0.24	18.17^{b}±0.71	24.33^{a}±0.00		NS	3.59	NS
30 DAS/Y1	12.33±0.82	14.33±3.19	17.00±3.24	20.00±2.55	25.33±4.81	17.80±2.55			
30 DAS/Y2	15.67±3.49	15.33±2.48	21.00±3.74	20.33±3.63	24.67±2.86	19.40±1.96			
Mean	14.00^{c}±2.36	14.83^{c}±0.71	19.00^{b}±2.83	20.17^{b}±0.24	25.00^{a}±0.47		NS	3.70	NS

DAS (days after sowing); LSD (least significant difference); Y (year); T (treatment); Cell values are given as mean of five replications; Mean values followed by different letters are significantly different within a row.

Table 3. Effect of *Rhizobium* inoculation and potassium application on protein content in leaves and nodules of lentil.

Stage /Year	Treatments						LSD at 5%		
	R_0K_0	R_1K_0	R_1K_{50}	R_2K_0	R_2K_{50}	Mean	Y	T	Y× T
	Protein content of leaves (mg g^{-1} FW)								
30 DAS/Y1	7.77±0.71	8.27±0.70	8.63±0.70	9.42±0.69	9.69±0.70	8.76±0.40			
30 DAS/Y2	8.40±0.81	8.92±0.69	9.23±0.77	10.06±0.69	10.26±0.73	9.38±0.39			
Mean	8.09^{d}±0.44	8.60^{cd}±0.46	8.93^{bc}±0.42	9.74^{ab}±0.46	9.98^{a}±0.40		NS	0.824	NS
30 DAS/Y1	10.21±1.45	10.62±1.39	10.80±1.32	11.77±1.67	12.21±1.74	11.12±0.42			
30 DAS/Y2	11.38±1.49	11.76±1.45	11.91±1.37	13.18±1.74	13.54±1.72	12.35±0.47			
Mean	10.80±0.83	11.19±0.80	11.36±0.78	12.47±1.00	12.87±0.94		NS	NS	NS
30 DAS/Y1	9.57±1.33	10.20±1.37	10.43±1.37	11.15±1.59	11.56±1.87	10.58±0.39			
30 DAS/Y2	10.62±1.32	11.33±1.38	11.54±1.39	12.47±1.55	13.05±1.85	11.80±0.48			
Mean	10.10±0.74	10.76±0.80	10.99±0.78	11.81±0.94	12.30±1.05		NS	NS	NS
	Protein content of nodules (mg g^{-1} FW)								
30 DAS/Y1	28.17±0.58	29.04±0.78	29.29±0.81	30.88±0.89	31.27±0.92	29.73±0.65			
30 DAS/Y2	28.67±0.64	29.71±0.86	29.97±0.81	31.58±0.92	32.03±0.93	30.39±0.69			
Mean	28.42^{c}±0.36	29.38^{b}±0.47	29.63^{b}±0.48	31.23^{a}±0.49	31.65^{a}±0.54		NS	0.939	NS
30 DAS/Y1	30.87±0.45	31.38±0.49	31.58±0.50	33.30±0.92	33.54±0.96	32.13±0.60			
30 DAS/Y2	31.23±0.47	31.73±0.52	32.04±0.53	34.12±0.95	34.27±0.89	32.68±0.71			
Mean	31.05^{b}±0.25	31.55^{b}±0.25	31.81^{b}±0.33	33.71^{a}±0.58	33.90^{a}±0.52		NS	0.802	NS
30 DAS/Y1	21.27±0.51	22.13±0.48	22.39±0.44	25.21±0.32	25.48±0.37	23.30±0.96			
30 DAS/Y2	21.70±0.58	22.49±0.40	22.73±0.41	25.52±0.38	25.83±0.41	23.65±0.94			
Mean	21.48^{c}±0.30	22.31^{b}±0.25	22.56^{b}±0.24	25.37^{a}±0.22	25.66^{a}±0.25		NS	0.499	NS

DAS (days after sowing); LSD (least significant difference); Y (year); T (treatment); Cell values are given as mean of five replications; Mean values followed by different letters are significantly different within a row.

Leghemoglobin content

Potassium application and *Rhizobium* inoculation increased leghemoglobin content of nodules in both growing seasons, showing significant differences ($P < 0.05$) from the control (R_0K_0). The highest leghemoglobin content was recorded with R_2K_{50} and the enhancement was highest at 60 DAS in both years of study. Lb content decreased towards physiological maturity. Lowest leghemoglobin content (0.128 mM) in nodules was recorded for R_0K_0 (30 DAS; Year 1) and highest leghemoglobin content (0.278 mM) was recorded for R_2K_{50} (60 DAS; Year 2). Leghemoglobin content increased by 8.34% and 9.54% at 60 DAS stage with R_2K_{50} in Year 1 and Year 2, respectively (Table 4).

Table 4. Effect of *Rhizobium* inoculation and potassium application on leghemoglobin content in nodules of lentil.

Stage/ Year	Treatments						LSD at 5%		
	R_0K_0	R_1K_0	R_1K_{50}	R_2K_0	R_2K_{50}	Mean	Y	T	Y×T
	Leghemoglobin content of nodules (mM)								
30 DAS/Y1	0.128±0.001	0.131±0.002	0.132±0.001	0.137±0.002	0.138±0.001	0.133^{b}±0.00			
30 DAS/Y2	0.138±0.002	0.143±0.003	0.145±0.002	0.149±0.002	0.151±0.002	0.145^{a}±0.00			
Mean	0.133^{c}±0.007	0.137^{b}±0.008	0.139^{b}±0.009	0.143^{a}±0.009	0.144^{a}±0.009		0.003	0.002	NS
30 DAS/Y1	0.250±0.003	0.251±0.003	0.252±0.003	0.270±0.005	0.271±0.005	0.259^{b}±0.005			
30 DAS/Y2	0.254±0.003	0.257±0.004	0.258±0.006	0.277±0.007	0.278±0.008	0.265^{a}±0.006			
Mean	0.252^{c}±0.003	0.254^{b}±0.004	0.255^{b}±0.004	0.273^{a}±0.005	0.275^{a}±0.005		0.009	0.005	NS
30 DAS/Y1	0.208±0.002	0.210±0.002	0.211±0.002	0.222±0.004	0.224±0.004	0.215±0.004			
30 DAS/Y2	0.210±0.003	0.211±0.002	0.213±0.003	0.223±0.005	0.225±0.005	0.216±0.003			
Mean	0.209^{b}±0.002	0.210^{b}±0.001	0.212^{b}±0.001	0.222^{a}±0.001	0.224^{a}±0.001		NS	0.003	NS

DAS (days after sowing); LSD (least significant difference); Y (year); T (treatment); Cell values are given as mean of five replications; Mean values followed by different letters are significantly different within a row.

Quality attributes

As evident from Table 5, K and *Rhizobium* had a profound effect on quality attributes of the crop. The trend of plant response to K and *Rhizobium* treatments relative to N and K content of seeds was almost similar to those observed for the other growth characteristics. R_2K_{50} was found to exhibit higher N and K content than control (R_0K_0). Potassium content of seeds increased from 1.06 (R_0K_0) to 1.40 (R_1K_{50}) and further to 1.93 (R_2K_{50}) in Year 1 and from 1.10 (R_0K_0) to 1.54 (R_1K_{50}) and further to 2.27 (R_2K_{50}) in Year 2. Potassium fertilization and *Rhizobium* inoculation significantly improved N and K concentrations and the highest values were obtained for the treatment R_2K_{50} in both years of study. The effect of K and *Rhizobium* interaction on seed protein content was significant (Table 5). Single application of *Rhizobium* as well as its combination with K significantly improved the protein content of seeds. The highest improvement was recorded with R_2K_{50} treatment in both years of study.

Table 5. Effect of *Rhizobium* inoculation and potassium application on quality attributes of lentil.

Year	Treatments						LSD at 5%		
	R_0K_0	R_1K_0	R_1K_{50}	R_2K_0	R_2K_{50}	Mean	Y	T	Y×T
	Seed K content (%)								
Year1	1.06±0.07	1.20±0.05	1.40±0.14	1.73±0.31	1.93±0.39	1.46±0.18			
Year2	1.10±0.06	1.27±0.10	1.54±0.18	2.02±0.36	2.27±0.41	1.64±0.25			
Mean	1.08^{d}±0.03	1.23^{cd}±0.05	1.47^{c}±0.10	1.88^{b}±0.21	2.10^{a}±0.25		NS	0.283	NS
	Seed N content (%)								
Year1	3.52±0.30	3.54±0.30	3.57±0.29	3.80±0.21	3.83±0.19	3.65±0.08			
Year2	3.76±0.30	3.78±0.31	3.81±0.29	3.97±0.20	3.99±0.20	3.86±0.05			
Mean	3.64±0.17	3.66±0.17	3.69±0.17	3.88±0.12	3.91±0.11		NS	NS	NS
	Seed protein content (%)								
Year1	21.98±1.88	22.10±1.87	22.33±1.80	23.73±1.28	23.96±1.21	22.82±0.47			
Year2	23.48±1.89	23.63±1.92	23.80±1.78	24.81±1.25	24.93±1.26	24.13±0.34			
Mean	22.73±1.07	22.87±1.08	23.07±1.04	24.27±0.76	24.44±0.69		NS	NS	NS

LSD (least significant difference); Y (year); T (treatment); Cell values are given as mean of five replications; Mean values followed by different letters are significantly different within a row.

Yield and yield components

Lentil variety under investigation responded significantly to K and *Rhizobium* application relative to number of pods plant^{-1} and 1000-seed weight (Table 6). The highest value obtained for number of pods was 69 and 94 with R_2K_{50} treatment in Year 1 and Year 2, respectively. Thousand seed weight was higher with R_2K_{50} and it was 23.18 g in Year 2 of study, exhibiting 33.42% increase over the untreated control (R_0K_0). The seed yield was higher with applied K and *Rhizobium* application as compared with control (R_0K_0). Significant differences existed among treatments applied. Seed yield in Year 1 of the study increased to 1528 kg ha^{-1} (R_2K_{50}) from 1109 kg ha^{-1} (R_0K_0), which was an increase of 38%. In Year 2, the yield increased from 1152 kg ha^{-1} (R_0K_0) to 1551 kg ha^{-1} (R_2K_{50}), showing an increase of 35% over the control (Table 6).

Table 6. Effect of *Rhizobium* inoculation and potassium application on yield characteristics and seed yield of lentil.

Year	Treatments						LSD at 5%		
	R_0K_0	R_1K_0	R_1K_{50}	R_2K_0	R_2K_{50}	Mean	Y	T	Y×T
	Number of pods (plant^{-1})								
Year1	46.33±2.94	51.33±2.86	57.00±4.30	63.00±3.54	68.67±3.19	57.27^{b}±4.46			
Year2	57.33±2.86	63.67±4.32	72.33±2.94	83.00±3.94	93.67±4.32	74.00^{a}±7.31			
Mean	51.83^{e}±7.78	57.50^{d}±8.72	64.67^{c}±10.84	73.00^{b}±14.14	81.17^{a}±17.68		5.75	3.64	NS
	1000 seed weight (g)								
Year1	16.72^{g}±0.52	17.74^{ef}±0.18	18.98^{d}±0.12	19.50^{cd}±0.21	20.10^{c}±0.34	18.61^{b}±0.68			
Year2	17.38^{fg}±0.41	18.59^{e}±0.19	19.71^{c}±0.41	22.11^{b}±0.41	23.18^{a}±0.58	20.19^{a}±1.21			
Mean	17.05^{e}±0.46	18.16^{d}±0.60	19.34^{c}±0.52	20.80^{b}±1.84	21.64^{a}±2.18		0.656	0.415	0.928
	Seed yield (g plant^{-1})								
Year1	3.20±0.14	3.77±0.15	4.07±0.17	4.20±0.15	4.47±0.04	3.94±0.24			
Year2	3.30±0.20	3.90±0.19	4.30±0.18	4.40±0.16	4.58±0.14	4.10±0.26			
Mean	3.25^{d}±0.07	3.84^{c}±0.09	4.19^{b}±0.16	4.30^{b}±0.14	4.53^{a}±0.08		NS	0.171	NS
	Seed yield (kg ha^{-1})								
Year1	1109.00±74.34	1305.33±55.95	1422.00±57.08	1474.33±55.86	1528.33±46.60	1367.80±83.27			
Year2	1151.67±84.84	1364.33±57.10	1439.67±72.91	1504.33±71.63	1551.33±50.92	1402.27±78.37			
Mean	1130.33^{d}±30.17	1334.83^{c}±41.72	1430.83^{b}±12.49	1489.33^{ab}±21.21	1539.83^{a}±16.26		NS	71.29	NS

LSD (least significant difference); Y (year); T (treatment); Cell values are given as mean of five replications; Mean values followed by different letters are significantly different within a row.

Discussion

In an effort to improve plant growth and productivity of lentil by increasing the amount of N_2-fixation, *Rhizobium* inoculation was supplemented with K application. As for the results, all observed parameters were higher in Year 2 (2009) than Year 1 (2008) of study. This can be attributed to the favorable climatic conditions in 2009 relative to 2008.

Greater plant height was observed in response to inoculation by the two *rhizobial* strains. Application of inoculums along with K fertilization further improved plant height. In this connection, K deprivation was reported to decrease plant growth characteristics in *Hordeum maritimum* (Hafsi *et al.*, 2011). Potassium fertilization resulted in vigorous and taller plants in our earlier study on *Ruta graveolens* (Malik *et al.*, 2012). *Rhizobium* forms nodules on the roots of legumes. Nodules are the sites of N fixation. *Rhizobia* are usually present in native soils but are either few in numbers or have limited nodulation capability and less N_2-fixation efficiency. Therefore, seeds of legumes are frequently inoculated with a specific *Rhizobium* strain to get better nodulation and greater N_2-fixation. Although, lentil is capable of fixing atmospheric N, under our agro-ecological conditions, nodulation of lentil is poor and is a major cause of its lower yield. Inorganic fertilizer treatments increase the N_2-fixation ability of root nodules in legumes as compared to the unfertilized plants on nutrient deficient soils. It has been argued that plant nutrients have an indirect role in N_2-fixation as they increase total leaf area and hence, photosynthesis. Progressive translocation of photosynthates towards roots improves root nodules, both in size as well as in number. In the present study, optimum K application improved nodulation of lentil inoculated with *rhizobia* strains probably by the above indirect approach. Roots of *Trifolium pratense* and *Medicago sativa* were found to have higher starch and sucrose contents when supplied with optimum levels of K than the plants supplied with low levels of K (Mengel *et al*., 1974). Availability of K influenced the efficiency of *Rhizobium* for the crop and improved its nodulation potential. This might have enhanced N_2-fixation resulting in better leaf area and photosynthesis, a *vice-versa* relationship.

Potassium plays important role as an activator of enzymes that are essential for the production of proteins and sugars. Soluble protein content in leaves and nodules was increased by K supplementation in our study. As per literature protein synthesis and enzyme activation required adequate amount of K^+ (Marshner, 1995). The significant effect of K application might be due to better K utilization which improves the uptake and assimilation of N to end up with a greater protein synthesis.

Lentil, being a legume, fixes atmospheric N in symbiosis with *rhizobia* present in the nodules on roots and converts it to an available form by Lb (a red pigment). Plants differ significantly in Lb content of nodules and bio-fertilizers have a major role in it. Increased Lb concentrations and nutrient uptake including that of Fe have been reported in lentil by single inoculation of *R. leguminosarum* as well as by dual inoculation of *R. leguminosarum* + *Pseudomonas* sp. (Mishra *et al*., 2011). It has been reported earlier in cowpea and soybean that nitrogenase activity increases, if Lb concentrations are high (Dakora, 1995), which finally increase above ground N partitioning to the seeds. The Lb content was estimated from fresh nodules and a significant increase was observed in it. This increase under K application and *Rhizobium* inoculation might be due to their role in the availability of some elements like Fe to the root nodules resulting in the enhancement of Lb content and finally into an increase in the N content of various plant organs including seeds.

The mean K contents (1.46% and 1.64% in Year 1 and Year 2, respectively) obtained in the present study were higher than reported in other studies (Petterson *et al*., 1997; Wang and Daun, 2006). Different plants have differential K utilization efficiency and differ in their ability to translocate K from vegetative organs to reproductive organs and finally to seeds. This has an important role in the economic yield of the plants. If the soil is deficient in K, there is a reduction in K concentration in the plant as has been reported by Hafsi *et al*. (2011) in *Hordeum maritimum*. As regards the percentage of N in seeds, Sangakkara *et al*. (1996) reported increased total N concentrations in *Vicia faba* and *Phaseolus vulgaris* by K application. The percentage of N in seeds was significantly affected in this study and it correlated with number of nodules. Bacterial inoculation and K application resulted in higher number of nodules which may have resulted in higher biological N fixation and hence greater translocation of N to seeds. Potassium nutrition has been associated with grain quality and protein content in various crops (Tiwari *et al*., 2012). This might be due to its role in enhancing N use efficiency and translocation of the biologically fixed N. Interactive effect of K and *Rhizobium* was more pronounced on protein content. This could be due to enhanced N_2-fixation by inoculated plants and subsequent translocation of the biologically fixed N to the seeds.

The two *rhizobia* strains resulted in higher number of pods and 1000 seed weight in both growing seasons. These results are in line with those of Hoque and Haq (1994). In this connection, Krishnareddy and Ahlawat (1996) reported that greater portion of the photo-assimilates was translocated to yield components than to biomass production under seed inoculation. Plant growth characteristics, protein content of nodules and leaves, leghemoglobin content as well as quality parameters were all influenced in positive direction by dual application of K and *Rhizobium*, resulting in the enhancement in yield attributes of the plant under study. Seed yield (g $plant^{-1}$ and kg ha^{-1}) was affected by *Rhizobium* inoculation and fertilizer applications probably due to their positive influence on growth and yield attributes. R_2K_{50} produced more pods per plant with highest 1000 seed weight and resulted in highest grain yield. These results are in line with those of Bremer *et al.* (1990), who reported that inoculation alone increased seed yield by 135%. Huang and Erickson (2007) also reported yield enhancement by inoculation in lentil. Therefore, *Rhizobium* inoculation and K application enhances N nutrition by improving nodulation and N fixation, and improves translocation of photosynthates resulting in improvement in growth attributes and enhancement in yield. Hence, we finally reveal that K application influences *Rhizobium* association and results in increase in the leghemoglobin content of nodules which in turn positively influences growth characteristics, protein content of nodules and other plant parts, quality parameters as well as yield attributes resulting finally in the yield enhancement of the crop.

Conclusion

The study revealed that lentil responds positively to *Rhizobium* inoculation in terms of plant growth characteristics, protein content of various organs, leghemoglobin content of nodules and quality as well as yield parameters. Among the *rhizobial* strains, L-1897 responded better than L-2097 in increasing the above parameters as well as yield of lentil variety L-4076. Potassium application along with *rhizobial* strains further augmented these parameters and increased seed yield to its highest value in this study. The yield obtained in this study is much more than the average yield reported from India for the two years (i.e., 2008 and 2009). Dual application of *Rhizobium* and K reduced the requirement of other fertilizers, particularly N for the lentil variety under study, mainly due to the positive effect of inoculation in increasing the root infectivity; hence number of nodules, and leghemoglobin content which finally increases biological N_2-fixation and therefore reduces the requirement of fertilizer N. From the present study, we conclude that K application positively influences *Rhizobium* inoculation and the association favors yield in upward direction and hence fulfilling the aim of the research. This combination is also a way to achieve sustainable and environment friendly agricultural production, and as a means to save the micro-ecosystems of soils from damage due to excessive use of chemical fertilizers.

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