Symbiotic Nitrogen Fixation, Growth and Yield Response of Common Bean and Cowpea for Rhizobia Inoculation and Micronutrients (B and Mo) Application in Mid-hill Regions of Nepal

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Abstract

Introduction

Due to the facts that legumes are capable of fixing atmospheric nitrogen through symbiotic nitrogen fixation (SNF) by root nodules inhabiting rhizobia and producing nitrogen (N) rich biomass/seeds, they are important for human and livestock to provide high quality protein sources (Foyer et al., 2016; Leigh, 2004). Legume crops widely used in intercropping/mixed cropping systems (Chapagain and Riseman, 2014; Thilakarathna et al., 2016), crop rotations (Hauggaard-Nielsen et al., 2008) and deposit fixed nitrogen into soil as root exudates (Fustec et al., 2010) as well as after litter decomposition (Thilakarathna et al., 2016), thus reduce the need for synthetic N fertilizers. However, some legume species and varieties suffer from sub-optimal SNF due to unavailability of compatible rhizobia strains in the soil, incompatible interactions with available soil rhizobia strains, competition by local ineffective rhizobia strains, adverse climatic conditions (e.g. drought), unfavorable soil conditions (e.g. salinity) and some nutrient deficiency in soil (e.g. micronutrients) (Giller, 2001; Thilakarathna and Raizada, 2017; Zahran, 1999). Even though compatible rhizobia strains are introduced to crop fields, they may not survive in the soil due to adverse soil and environmental conditions (Thilakarathna and Raizada, 2017). This may reduce the SNF potential of the legume crops, thus repeated inoculation of rhizobia is necessary during next growing seasons.

Grain legumes play a very important role in providing high quality protein sources for millions of people in the world, especially in developing nations (Foyer et al., 2016). Nepal, as a developing country, grows grain legume on over 334,323 ha of land with a total production of 319,770 metric tons (i.e., ~1t/ha) of which lentil (*Lens culinaris* Medik.) accounts for >60% of area and production emerging as an important export commodity (Gharti et al., 2014). The other legumes of economic importance include: common bean (*Phaseolus vulgaris* L.), chickpea (*Cicer arietinum* L.), pigeonpea (*Cajanus cajan* L. Millspaugh), soybean (*Glycine max* L. Merr.), mungbean (*Vigna radiate* L. Wilczek), blackgram (*Vigna mungo* L. Hepper), field pea (*Pisum sativum* L.), horsegram (*Macrotyloma uniflorum* Lam. Verdc.), and cowpea (*Vigna ungiculata* L. Walpers), resulting in development of 35 improved varieties and a dozen of production technologies for different agro-ecological domains (Chapagain and Raizada, 2017; Gharti et al., 2014). These crops are mostly grown as sole crop depending on the season and location (Chapagain and Gurung, 2010; Chapagain and Raizada, 2017). For example, in the mid-hill regions of Nepal, cowpea is grown during spring-summer (i.e., March/April-July/August) while soybean, common bean, blackgram and horsegram are mostly grown during summer-rainy season (i.e., July/August-November/December). The winter season legumes (November/December-March/April) include lentil, chickpea and pea.

Challenges associated with legume production in Nepal include: lack of high yielding varieties and crop specific management (e.g., disease/pest management) practices, loss of soil fertility including micronutrients, poor access to agricultural inputs and services, shortage of irrigation water, lack of mechanization, and labour shortages which led to poor yields and low economic returns to farmers (Chapagain and Gurung, 2010; Chapagain and Raizada, 2017). Farmers mostly used untreated seeds from previous season harvest to cultivate these crops and very little attention has been given to study the effects of bacterial inoculation on the crop performance and soil health through systemic on-farm trials. Symbiotic nitrogen fixation of legumes can be improved by introducing efficient rhizobia strains for N fixation (Thilakarathna and Raizada, 2017), and also through application of soil deficient micronutrients which are important for different stages of SNF (Weisany et al., 2013). This research explores the opportunities to use different rhizobia strains (native as well as exotic) along with micronutrients (Boron and Molybdenum) on two legume crops (e.g., cowpea and common bean) to enhance SNF, crop yields (grain and plant biomass), plant and grain N content, and offers the most productive combination(s) for each crop.

Materials and Methods

Study site, climate and soil:

This study was conducted in two mid-hill districts of Nepal namely, Dhading and Kaski, for two rotation cycles from 2015 to 2017. The experimental sites in Dhading were located at 27° 78' 84" N and 84° 70' 02" E, at an altitude of 700-1300 m above mean sea level (masl) while the sites in Kaski were situated at 28° 20' 25" N and 84° 11' 71" E, at an altitude of 1100 masl. Research was conducted at farmer's fields under natural climatic conditions.

Climatic data for the experiment were collected from a regional weather station at the research site (Figure 1). Average day-time temperature over the cropping seasons (August – December, 2015/16) was 22^0C in Dhading and 20.4^0C in Kaski with the warmest days in August in both sites. Both Dhading and Kaski received more rainfall (seasonal total of 1107 mm and 1610 mm, respectively) in 2016; however, they received no or very little rains in October-December (Figure 1).

The soil was moderately well drained coarse textured sandy loam with low to moderate fertility. Soil samples were collected (0-20 cm) from farmer's field in each testing sites at the time of plot establishment. The soil was analyzed for pH (using a soil water solution of 1:2.5 wt/v), organic matter (Walkley-black method), total N (Modified Kjeldahl method), available P (Bray-P1 method) and available K (flame photometer with 1M ammonium acetate extracting solution) (Anderson and Ingram, 1993), and showed acceptably homogeneous conditions. The average pH, organic matter content, total N, available P_2O_5 and K_2O in Dhading were 6.1, 282 g kg^{-1} dry soil, 2.24 g kg^{-1} dry soil, 60.7 mg kg^{-1} dry soil and 146.4 mg kg^{-1} dry soil, respectively while these values were 5.48, 314 g kg^{-1} dry soil, 2.44 g kg^{-1} dry soil, 58 mg kg^{-1} dry soil and 114.9 mg kg^{-1} dry soil in Kaski. The site was used for grain (maize-millet-beans) production in prior years and managed by using farm yard manures (FYM), very small amount chemical fertilizer (i.e. urea) and no plant protection compounds.

Treatment details: Trials were conducted in 10 farmer's fields in each site for each crop. The experimental design consisted of five (T1-T5) treatments in 2015 and six treatments (T1-T6) in 2016, T1; not inoculated with rhizobia (i.e., control), T2; micronutrient treatment [boron (B) + molybdenum (Mo)], T3; inoculated with locally isolated indigenous rhizobia, T4; inoculated with reference rhizobia strain (USDA 9030, TTC9), T5; inoculated with reference rhizobia strain + B + Mo, T6; inoculated with local rhizobia + B + Mo. Treatments were allocated in randomized complete block design (RCBD). Treatments were replicated across farmers' fields but not within each farmer's field.

Plant materials, rhizobium strains and inoculum preparation

Local Nepalese varieties of common bean (*Phaseolus vulgaris* cv. Chaumase Simi) and cowpea (*Vigna ungiculata* cv. Malepatan) were used in both sites for two production years. Local indigenous rhizobia were isolated from root nodules of locally grown bean and cowpea according to the standard protocol (Somasegaran and Hoben 2012). Reference rhizobia strains for common bean and cowpea were *Rhizobia tropici* USDA 9030 (van Berkum et al., 1996) and *Bradyrhizobium yuanmingense* TTC9 (Sarr et al., 2009), respectively. Rhizobia strains of common bean (USDA 9030 and local rhizobia) and cowpea (TTC9 and local rhizobia) were grown in yeast mannitol agar (YMA) and modified arabinose-gluconate medium (MAG) respectively as liquid culture for 5 days at 28 °C. To prepare each inoculum, final cell density was adjusted to $OD_{595} = 1$ and uniformly mixed with finely ground press mud. According to the treatments, seeds were inoculated with rhizobia inoculants at a rate of 10 g inoculant per 100 g of seeds. Gum arabic solution (40% wt/vol) was used (2 ml/100 g seeds) as an adhesive agent to assure that the rhizobia inoculants coated well around the seeds. Inoculation of seeds was carried out in the field before planting and the treated seeds were stored under shade until seeding.

***Planting and application of micronutrients* (*B and Mo*)**

Plots were prepared along the terraces. Each plot measured 5 m x 6 m for common bean and 5 m x 4 m for cowpea. Seeds were planted at 80 cm (row spacing) x 40 cm (within row) in common bean and 60 cm x 30 cm in cowpea. In 2015, common bean and cowpea were seeded during 23-28 Aug in Kaski and 02-10 Sept in Dhading. In 2016, seeding was carried out during 18-23 Aug and 14-26 Aug in Kaski and Dhading, respectively. Micronutrients (B and Mo) were side dressed one week after germination at the rate of 300 g B per ha (2 kg borax per ha) and 150 g Mo per ha (326 g sodium molybdate per ha) (Plant-Prod ON, Canada) according to the treatments. They were applied to both sides of the plants, 10 cm away from the stem and 5 cm deeper into the soil. Bamboo sticks were used as trellis for common bean. The common aftercare management practices including weeding were carried out according to the local farmer practices and managed equally across treatments.

Sample collection

Plant samples were taken at flowering stage and maturity. At flowering stage, five plants were randomly collected from middle of each subplot for nodule number, nodule dry weight and shoot and root dry weight (DWT). Dry weight of tissue samples was determined after putting samples for 72 hours in an oven at 65 ^{o}C. Dried shoot samples were ground and analyzed for total N (%) according to the modified Kjeldahl method (Anderson and Ingram, 1993). Total N content of shoot materials was calculated by multiplying shoot dry weight by shoot N (%). At maturity, seeds were collected from 3m x 3m area, where five plants from each sub plot were collected to measure shoot and root dry weight. Seed samples were dried and moisture content was determined. Final seed weight was expressed at 13% moisture content. Seed samples from 2016 were analyzed for total N (%) according to the modified Kjeldahl method. Seed N content was calculated by multiplying seed dry weight at 13% moisture content by seed N (%).

Statistical analysis

The data were analyzed as three factor factorial design (year x site x treatment) using analysis of variance (ANOVA) set at $p < 0.05$. Overall analysis outputs including the main effects (year, location, treatments) and their interactions are presented in Table 2. The effect of different rhizobia/micronutrient treatments at each location in each year was compared with Fisher least significant differences method. Shoot biomass at flowering, total shoot N at flowering, grain yield, grain N content versus nodule number and nodule dry weight correlation analysis was performed using the Pearson correlation test. All statistical analyses were performed using GraphPad Prism Software (v7, GraphPad Software, USA).

Results

Nodulation, biomass and plant N data at flowering stage – common bean

Rhizobium and micronutrient treatments showed positive effects on nodule number, nodule DWT, shoot DWT and shoot total N content at flowering (Table 2). However, their effects on root DWT and shoot C/N ratio at flowering were not significant (Table 2). In comparison to the uninoculated control, seed inoculation with rhizobia increased the nodule number (18-76%), nodule DWT (59-106%), shoot DWT (13-30%) and shoot total N content (13-30%) in 2015. During second production year (2016), application of micronutrients and/or rhizobia increased the nodule number (67-163%), nodule DWT (70-145%), shoot DWT (-1-26%) and shoot total N content (42-74%) compared to the control treatment. In 2015, nodule number and nodule dry weight were higher in Kaski compared to the Dhading (Fig. 1a,c), but opposite trend was found with shoot DWT, shoot total N content and shoot C/N ratio (Table 3). Generally, all the measured parameters were higher in Kaski in the second production year (2016) compared to the Dhading (Fig. 1b, 1d, Table 3). In Kaski, nodulation by native soil rhizobia was high (8 and 37 nodules per plant in 2015 and 2016, respectively) compared to Dhading (3 and 15 nodules per plant in 2015 and 2016, respectively).

Nodulation, biomass and plant N data at flowering stage – cowpea

In general, nodule number, nodule DWT, shoot DWT and shoot total N content of cowpea at flowering were not influenced by the rhizobia/ micronutrient treatments (Table 2). Average number of nodules and nodule dry weight varied by location (Table 2). The number of nodules in Dhading was very low (2-6 nodules plant^{-1}) compared to the Kaski (15-29 nodules plant^{-1}) in 2015 and 2016 (Fig. 3a,b). In Kaski, plants that were treated with micronutrients alone and reference strain + micronutrients had higher nodule numbers (26-29 nodules plant^{-1}) compared to the local rhizobia treatments (16 nodules plant^{-1}) in 2016 (Fig. 3b). In comparison to the Dhading, Kaski had higher nodule dry weight in 2015 (average 339% higher) and 2016 (average 482% higher) (Fig. 3c, d). Furthermore, in 2016, nodule dry weight was significantly lower than 2015 production year in Kaski (4.6 times lower) and Dhading (6.4 times lower) (Fig. 3c, d). On average, Dhading site had higher root (39%) and shoot (38%) dry weight compared to the Kaski (Table 4). Production year also had a significant effect on root and shoot DWT, where higher root (31%) and shoot (118%) dry weights were found in 2016 compared to the 2015 (Table 4). In comparison to the Kaski, shoot N content at flowering was higher in Dhading in 2015 (70.2%) and 2016 (30.4%). In general, shoot N content was much higher in 2016 (87-144%) compared to the 2015 production year (Table 4). Shoot C/N ratio was higher in Dhading (35.3%) compared to the Kaski in 2015, whereas opposite trend was found in 2016 (Table 4).

Grain yield and grain N data at harvest – common bean

Rhozobia and micronutrient treatments did not have a significant impact on grain yield of common bean (Table 2). However, rhizobia inoculation alone increased the yield by 26% (local rhizobia) and 41% (reference rhizobia) compared to the uninoculated control in 2015, whereas 30% yield improvement was found in 2016 with both the rhizobia strains. Application of micronutrient alone increased the yield by 31% in 2015 and 20% in 2016 compared to the uninoculated control. Treatments with rhizobia + micronutrients also improved the grain yield by 33% in 2015 and 29-37% in 2016 compared to the uninoculated control. Grain yield was different based on the year and location (Table 2), where higher grain yield was found in 2016 compared to the 2015 production year (Fig. 4a,b). Furthermore, grain yield production was higher in Kaski compared to the Dhading in 2015 (by 116%) and 2016 (by 64%) (Fig. 3a,b). Grain N% was higher with the reference rhizobia strain + micronutrient treatment (3.47% N) compared to the other five treatments (2.94-3.11% N) in 2016. Furthermore, grain N% varied by treatment by location effect (Table 2). In Kaski, plants inoculated with reference rhizobia + micronutrients had the highest grain N%, whereas plants that were received only micronutrient treatment had the lowest grain N% (Table 3). Similar to grain N%, total grain N content was higher in plants treated with reference strain + micronutrients compared to the other five treatments in Kaski (Fig. 4a). There was a significant location effect for grain N content (Table 2), where total grain N content (mg N plant^{-1}) was 46% higher in Kaski compared to the Dhading (Fig. 4a).

Biomass, yield and grain N data at harvest – cowpea

Rhizobia and micronutrient treatments did not have a significant impact on grain yield, grain N% or grain N content of cowpea (Table 2). However, during 2015 production year rhizobia/micronutrient treatments had positive effect on yield, where grain yield was increased with the local rhizobia (8%), reference rhizobia (14%) and reference rhizobia + micronutrients (31%) treatments compared to the uninoculated control (Fig. 4b). Grain yield of cowpea was varied by year by location effect (Table 2). Specially in 2016, higher grain yield was produced in Dhading (33.2%) compared to the Kaski (Fig. 4b). On average, grain yield was higher in 2016 (163%) compared to the 2015 (Fig. 4c,d). In comparison to Kaski, higher grain N% (24%) and grain N content (62%) were recorded in Dhading in 2016 (Table 4; Fig. 5b).

Discussion

Tables

Table 1 Soil type and chemical properties of the soil collected from farmers field during 2015 and 2016 across Kaski and Dhading sites from top 15-20 cm soil depth.

Crop	Year	Site	Soil type	pH	OM (%)	Total N (g Kg^{-1})	P (mg Kg^{-1})	K (mg Kg^{-1})
Common bean	2015	Kaski	Sandy loam	5.52	3.87	2.70	92.3	78.2
		Dhading	Sandy loam	6.66	3.39	2.35	47.4	163.0
	2016	Kaski	Sandy loam	6.10	2.13	2.33	52.9	164.1
		Dhading	Sandy loam	4.72	2.66	1.86	75.5	82.9
Cowpea	2015	Kaski	Sandy loam	5.43	3.71	2.35	23.3	95.1
		Dhading	Sandy loam	6.52	3.18	2.25	42.4	143.7
	2016	Kaski	Sandy loam	4.84	2.83	2.36	63.4	122.0
		Dhading	Sandy loam	6.51	2.06	2.50	77.3	195.9

Table 2 Summery of the effects of treatments, years and locations and their interactions on different parameters tested

Parameters	Treatment	Year	Location	Treatment x Year	Treatment x location	Year x Location	Treatment x Year x Location
Common bean							
Nodule #	*	****	****	ns	ns	****	ns
Nodule DWT	*	ns	****	ns	ns	****	ns
Root DWT	ns	***	****	ns	ns	*	ns
Shoot DWT	**	**	*	ns	ns	****	ns
Shoot total N	**	*	***	ns	ns	****	ns
Shoot C/N ratio	ns	*	ns	ns	ns	****	ns
Grain Yield	ns	****	****	ns	ns	ns	ns
Grain N (%)	**	–	*	–	*	–	–
Grain N content	ns	–	***	–	ns	–	–
Cowpea							
Nodule #	ns	ns	****	ns	ns	ns	ns
Nodule DWT	ns	****	****	ns	ns	****	ns
Root DWT	ns	****	****	ns	ns	ns	ns
Shoot DWT	ns	****	****	ns	ns	ns	ns
Shoot total N	ns	****	ns	ns	ns	****	ns
Shoot C/N ratio	ns	*	ns	ns	ns	****	ns
Grain Yield	ns	****	*	ns	ns	**	ns
Grain N (%)	ns	–	****	–	ns	–	–
Grain N content	ns	–	****	–	ns	–	–

All the units are per plant basis. DWT, dry weight. *, **, ***, **** Significant at the 0.05, 0.01, 0.001, and <0.0001 probability levels, respectively. ns, not significantly different at $P = 0.05$ level. −, not applicable.

Table 3 Effect of rhizobia inoculation and micronutrients (B + Mo) on root dry weight, shoot dry weight and shoot N content at flowering and root dry weight, shoot dry weight and grain N % at harvest in common bean at Kaski and Dhading sites during 2015 and 2016 cropping seasons.

Treatments/ Sites	**Root DW[1] (g plant^{-1})**		**Shoot DW[1] (mg plant^{-1})**		**Shoot total N[1] (mg N plant^{-1})**		**Shoot C/N ratio[1]**		**Grain N%[2]**
Kaski	2015	2016	2015	2016	2015	2016	2015	2016	2016
Uninoculated	0.96	0.85	4.23bc	5.81	148	179b	9.43	11.67	3.20ab
B + Mo	1.07	1.01	3.84c	7.32	129	269ab	10.44	9.74	2.64c
Local rhizobia (LR)	0.98	1.08	4.44bc	9.55	167	311a	9.10	10.82	3.06abc
9030	1.13	0.87	5.40ab	7.74	219	260ab	8.54	10.85	2.80bc
9030 + B + Mo	1.10	1.14	5.73a	9.70	213	331a	9.41	10.80	3.52a
LR + B + Mo	−	0.98	−	8.47	−	310a	−	9.62	2.96bc
Mean	1.05	0.99	4.73	8.10	175	277	9.38	10.58	3.03
P-value	ns	ns	0.0479	ns	ns	0.0489	ns	ns	0.0088
Dhading									
Uninoculated	0.80	0.66	6.88	7.85	226	72	11.55	8.87	3.03
B + Mo	0.76	0.60	5.75	6.24	190	88	11.07	8.96	3.44
Local rhizobia	0.96	0.71	8.17	4.92	240	112	12.95	8.25	3.09
9030	0.92	0.76	9.05	8.28	293	115	10.78	8.16	3.08
9030 + B + Mo	1.01	0.77	9.04	7.53	283	95	10.84	8.78	3.41
LR + B + Mo	−	0.94	−	6.93	−	124	−	8.71	3.05
Mean	0.83	0.74	8.05	6.96	246	101	11.77	8.62	3.18
P-value	ns	ns	ns	ns	ns	ns	ns	ns	ns

All the units are per plant basis. DW, dry weight. [1], at flowering stage. [2], at harvest. ns, not significantly different at $P < 0.05$ level. −, not applicable. Values followed by the same letter, within each column, are not significantly different at $P < 0.05$. n = 10

Table 4 Effect of rhizobia inoculation and micronutrients (B + Mo) on root dry weight, shoot dry weight and shoot N content at flowering and root dry weight, shoot dry weight and grain N % at harvest in Cowpea at Kaski and Dhading sites during 2015 and 2016 cropping seasons.

Treatments/ Sites	**Root DW[1] (g plant^{-1})**		**Shoot DW[1] (mg plant^{-1})**		**Shoot total N[1] (mg N plant^{-1})**		**Shoot C/N ratio[1]**		**Grain N%[2]**
Kaski	2015	2016	2015	2016	2015	2016	2015	2016	2016
Uninoculated	1.18	2.32	2.81	10.36	105	373	139	10.13	3.13
B + Mo	1.06	1.93	3.45	8.57	141	292	168	11.14	3.31
Local rhizobia (LR)	1.35	1.81	2.05	6.75	105	250	99	10.51	3.49
TTC9	1.23	2.11	2.86	8.85	119	303	115	11.13	3.33
TTC9 + B + Mo	1.33	2.29	2.93	9.38	144	355	135	9.755	3.55
LR + B + Mo	−	2.20	−	9.42	−	342	−	10.58	3.34
Mean	1.23	2.11	2.82	8.89	131	319	7.70	10.54	3.36
P-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dhading									
Uninoculated	1.92	2.44	6.13	10.24	244	393	10.52	9.53	4.10
B + Mo	2.24	2.27	6.21	10.24	206	407	11.17	9.81	4.16
Local rhizobia	1.82	2.58	4.64	10.24	178	404	9.93	9.83	4.11
Local rhizobia	2.16	2.25	6.99	10.24	271	355	8.98	9.01	4.23
TTC9	1.88	2.49	5.83	10.24	216	504	11.49	9.09	4.47
LR + B + Mo	−	2.25	−	10.24	−	436	−	9.15	3.97
Mean	2.00	2.38	5.96	10.24	223	416	10.42	9.40	4.17
P-value	ns	ns	ns	ns	ns	ns	ns	ns	ns

All the units are per plant basis. DW, dry weight. [1], at flowering stage. [2], at harvest. ns, not significantly different at $P < 0.05$ level. −, not applicable. n = 10

Figure Legends

Fig. 1 Average monthly temperature and total monthly precipitation at Kaski and Dhading, Nepal during 2015 and 2016**.**

Fig. 2 Nodule number (a,b) and nodule dry weight (c,d) of common bean at flowering stage in Kaski and Dhading during 2015 and 2016 seasons. Vertical bars indicates standard error of the mean. Bars followed by the same letter or without letter grouping are not significantly different at $P < 0.05$. n = 10

Fig. 3 Nodule number (a,b) and nodule dry weight (c,d) of cowpea at flowering stage in Kaski and Dhading during 2015 and 2016 seasons. Vertical bars indicates standard error of the mean. Bars followed by the same letter or without letter grouping are not significantly different at $P < 0.05$. n = 10

Fig. 4 Grain yield of common bean (a,b) and cowpea (c,d) in Kaski and Dhading during 2015 and 2016 seasons. Vertical bars indicates standard error of the mean. Bars followed by the same letter or without letter grouping are not significantly different at $P < 0.05$. n = 10

Fig. 5 Total grain nitrogen content of common bean (a) and cowpea (b) in Kaski and Dhading during 2016 season. Vertical bars indicates standard error of the mean. Bars followed by the same letter or without letter grouping are not significantly different at $P < 0.05$. n = 10

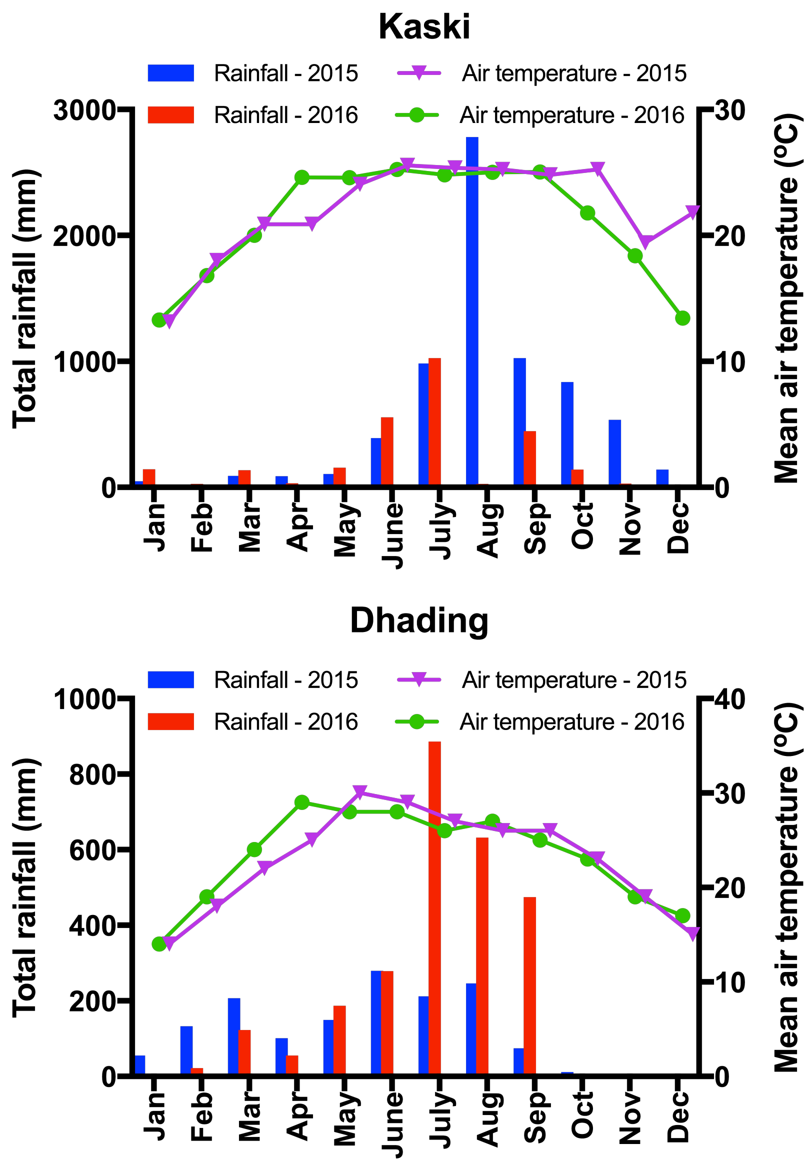
Kaski
Rainfall - 2015
Rainfall - 2016
Air temperature - 2015
Air temperature - 2016
Total rainfall (mm)
Mean air temperature (ºC)
3000
2000
1000
0
30
20
10
0
Jan
Feb
Mar
Apr
May
June
July
Aug
Sep
Oct
Nov
Dec
Dhading
Rainfall - 2015
Rainfall - 2016
Air temperature - 2015
Air temperature - 2016
Total rainfall (mm)
Mean air temperature (ºC)
1000
800
600
400
200
0
40
30
20
10
0
Jan
Feb
Mar
Apr
May
June
July
Aug
Sep
Oct
Nov
Dec

Fig. 1

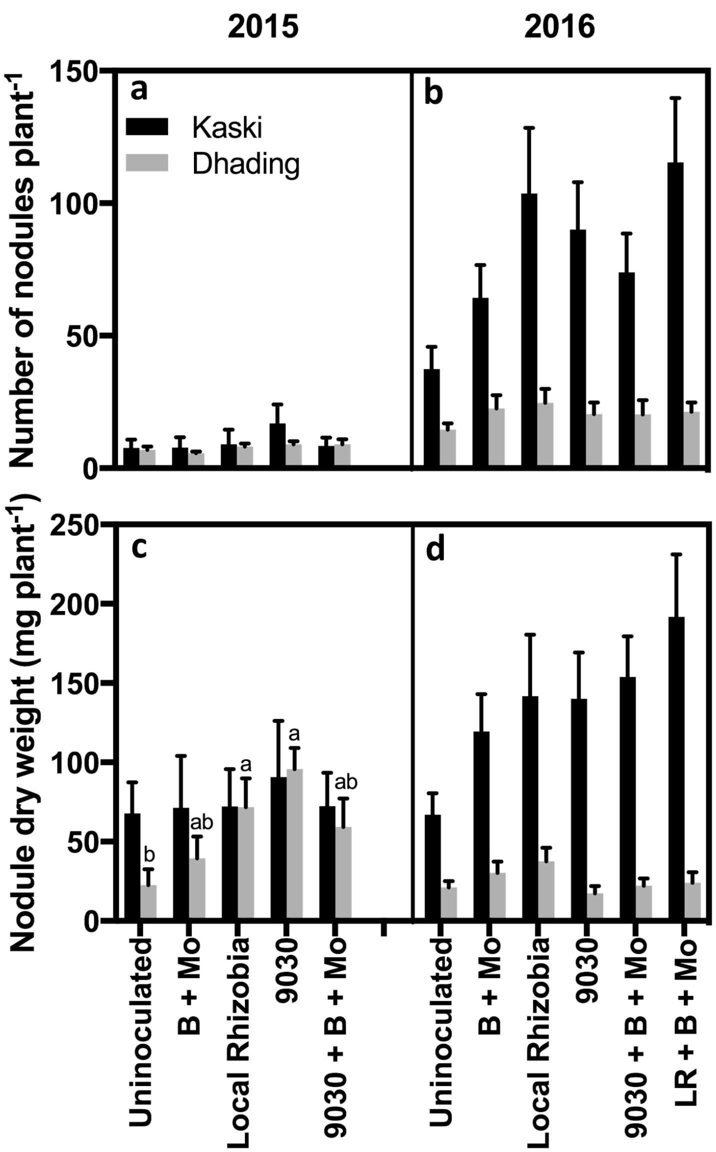
2015
2016
a
b
c
d
Kaski
Dhading
Number of nodules plant-1
Nodule dry weight (mg plant-1)
150
100
50
0
250
200
150
100
50
0
b
ab
a
a
ab
Uninoculated
B + Mo
Local Rhizobia
9030
9030 + B + Mo
Uninoculated
B + Mo
Local Rhizobia
9030
9030 + B + Mo
LR + B + Mo

Fig. 2

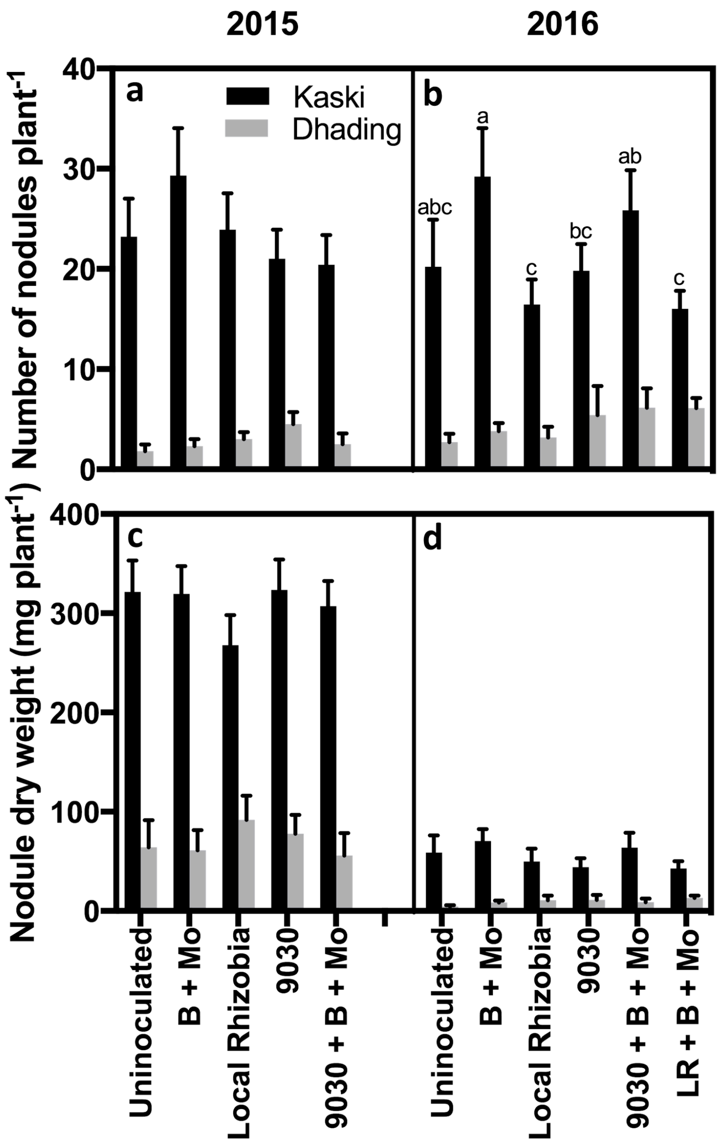
2015
2016
Number of nodules plant-1
Nodule dry weight (mg plant-1)
a
b
c
d
Kaski
Dhading
abc
a
c
bc
ab
c
40
30
20
10
0
400
300
200
100
0
Uninoculated
B + Mo
Local Rhizobia
9030
9030 + B + Mo
Uninoculated
B + Mo
Local Rhizobia
9030
9030 + B + Mo
LR + B + Mo

Fig. 3

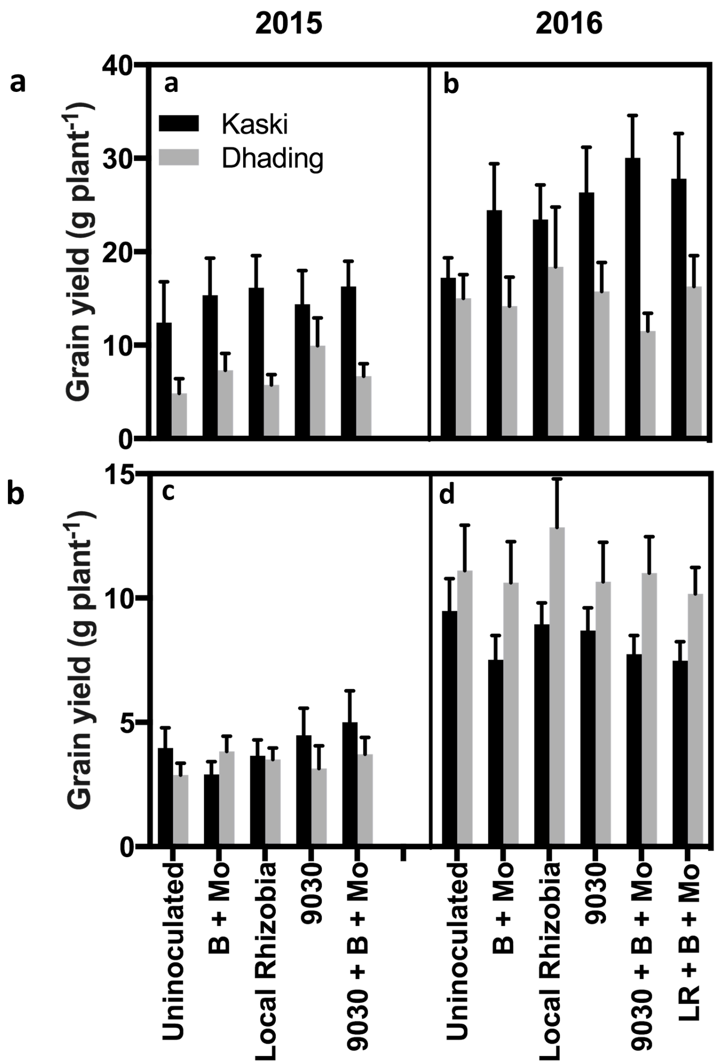
2015
2016
a
b
40
30
20
10
0
Grain yield (g plant-1)
Kaski
Dhading
15
10
5
0
c
d
Uninoculated
B + Mo
Local Rhizobia
9030
9030 + B + Mo
LR + B + Mo

Fig. 4

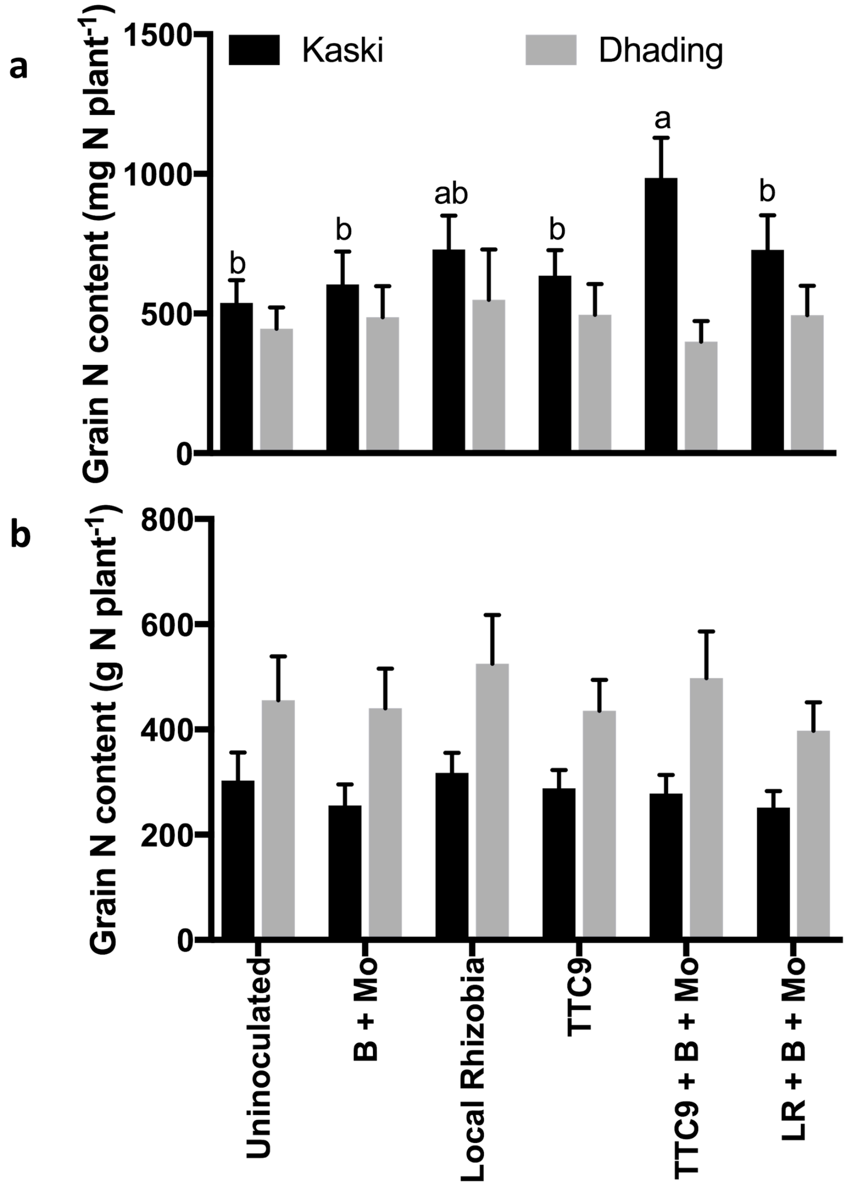
a
b
Kaski
Dhading
Grain N content (mg N plant^{-1})
Grain N content (g N plant^{-1})
1500
1000
500
0
800
600
400
200
a
ab
b
Uninoculated
B + Mo
Local Rhizobia
TTC9
TTC9 + B + Mo
LR + B + Mo

Fig. 5