The Journal of Animal & Plant Sciences, 28(5): 2018, Page: 1493-1500
ISSN: 1018-7081

THE EFFECT OF *RHIZOBIUM* SEED INOCULATION ON YIELDS AND QUALITY OF FORAGE AND SEED OF BERSEEM CLOVER (*TRIFOLIUM ALEXANDRINUM* L.) AND ITS IMPACT ON SOIL FERTILITY AND SMALLHOLDER FARMER'S INCOME

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ABSTRACT

A field experiment was conducted to investigate the effects of *Rhizobium* seed inoculation on forage and seed yield components and the forage quality of berseem clover (*Trifolium alexandrinum*). The experiment comprised of two treatments, seed inoculation with *Rhizobium trifolii* and the non-inoculation (control). The results revealed that the seed inoculation significantly affected (P<0.005) and produced maximum number of stems/m^2 (348.2), plant height (24.4cm), green forage yield (39.9 t/ha), dry matter yield (5.54 t/ha), number of heads per m^2 (339), number of seeds per head (24.5), 1000-seed weight (3.851 g) and predicted seed yield (320 kg/ha) as compared to non-inoculated plots. The similar trend of results was also found in the forage quality parameters and seed inoculation produced maximum values of neutral detergent fiber (26.5%), crude protein (28.6%) and water soluble carbohydrates (2.02%). However, the acid detergent fiber (21.0%) and metabolisable energy (10.7%) were found to be non-significant. The use of *Rhizobium* inoculum also added available nitrogen (N) to soil after the crop increasing available N to 0.0414% as compared to 0.0283% in the non-inoculated control. The use of *Rhizobium trifolii* inoculum in combination with the improved variety Agaitti Berseem-2002, produced an additional net income of PKR 111,913 Rs/ha (US$ 1145/ha) compared to non-inoculated plots.

Key words: Forage development, forage quality, poverty alleviation, rural development, seed production, smallholder farmers.

INTRODUCTION

Legume forages provide high quality feed for livestock and also play an important role in enhancing soil fertility by adding atmospheric N to the soil through bacterial N fixation (Muir *et al*., 2014). The inclusion of legume forages in the cropping system can lead to increased farm profitability and subsequently reducing the need for supplementary feeds in animal rations (Cosentino *et al*., 2014), thereby reducing expenses.

No data have been collected on the impact of seed inoculation on seed yield and forage quality factors in Pakistan. Berseem clover is less reliant on N from fertiliser sources because of the symbiotic relationship it shares with the bacteria *Rhizobium leguminosarum b.v. trifolii,* which provides an N source to the plant and subsequent crops (Graves *et al*., 1990, Cosentino *et al*., 2014). In Pakistan, inoculation of berseem clover seed has be shown to increase yields by greater than 10% (Naveed *et al*., 2015). The increased soil N levels as a result of N fixation can also benefit subsequent crops (Panciera and Sparrow, 1995, Agarwal and Ahmad, 2010) with yield increases of up to 13% reported in (subsequent) cereal and fibre crops such as wheat, rice, maize and cotton (Naveed *et al*., 2015). The potential improvements to berseem clover forage production from seed innoculation are well established (Bajpai *et al*., 1974, Oushy, 2008, El-Lithy *et al*., 2014), although most have only reported the impacts on green forage yield. Oushy (2008) also reported an increase in berseem clover forage protein levels with the use of seed inoculation and N fertiliser.

Berseem clover can fix between 115 to 400 kg N/ha in soil during its growing season, depending on the variety and season (Graves *et al*., 1990). However, berseem clover initially draws down soil N early in its life cycle for initial growth and development of plants (Oushy, 2008). Therefore, a basal application of N fertiliser is recommended to initiate growth and development of roots and shoots. The efficacy of N fixation is greatly dependent on *Rhizobium* activity, soil conditions and the variety of berseem clover grown (Radwan *et al*., 2006). In alkaline soils (pH>8), the availability of P can be limited due to its sequestration with Ca in the soils, causing a reduction in crop yields (Jan *et al*., 2014). Soil salinity can increase rapidly and have a drastic impact on plant growth (Agarwal and Ahmad, 2010). Salinisation is becoming an acute problem in agriculture in the study region due to the intensive cropping system and excessive use of salty tube well water for crop irrigation (Ghulam *et al*., 2013).

Rhizobia are very sensitive to salt stress and thus the impact of *Rhizobium* seed inoculation on the growth and yield of berseem clover also varies depending on the salinity of the irrigation water. Agarwal and Ahmad (2010) reported that using low salinity irrigation water (EC≤8 dS/m) produced the best responses to seed inoculation in berseem clover.

Inoculation not only influences berseem clover production but also contributes to the yields of the crops grown subsequently (Khan *et al*., 1985). Moreover, inoculation can be performed on different legume crops including pulses and the beneficial effects of inoculation have been found to be persistent more than 2 years post inoculation in low input agricultural systems, making it economically and ecologically sustainable (Pellegrino *et al*., 2011). Despite the obvious benefits, the inoculation of berseem clover forage crops is currently not being practiced in smallholder production systems in Pakistan due to lack of awareness (ignorance) of farmers. Furthermore, inoculums are often not available in the agricultural markets and when they are, they have not been appropriately stored (Naveed *et al*., 2015), primarily because of electricity shortage in the country.

This study was designed to investigate the impacts of seed inoculation on forage and seed yields and forage quality of berseem clover. Further, as the N fixation potential can be affected by variety, the opportunity was taken to gather data for the new variety of berseem clover (Agaitti Berseem-2002). Results were extrapolated to investigate the potential income benefits from using seed inoculation.

MATERIALS AND METHODS

The field experiment was conducted at University of Veterinary and Animal Sciences (UVAS) Ravi campus-Pattoki, Pakistan during the 2013-2014 growing season. The experimental site was located at 31º 03' 34.8''N and 73º 52' 42.6''E in the Kasur district of Punjab, Pakistan. The soil analysis results showed that the research site characterised as loamy soil with 0.45% organic matter, having a pH ($CaCl_2$) of 9.1 and EC value of 5.3 dS/m.

Seed inoculation: Agaitti Berseem-2002 variety seed was inoculated with viable Rhizobium trifolii (sourced from the Ayub Agricultural Research Institute (AARI), Faisalabad Punjab, Pakistan) that had been stored at 28 ± 2°C. The inoculum was used at the rate 250 g/ha (2.5 bacteria culture bags/ha) for 20 kg of seed, as one culture bag (containing 100 g of culture) was recommended for 1 acre (0.40 ha) (Qureshi *et al*., 2012). The culture was mixed into a 10% sugar solution to make a slurry for coating (to achieve better adhesiveness) and then sprinkled over the seeds and mixed thoroughly, ensuring that the culture coated all of the seed. Treated seed was left to stand under shade for 2 h before sowing as per the Research Institute recommendations, and as described by Agarwal and Ahmad (2010).

Experimental design: The experiment was laid out in a randomised complete block design at UVAS-Pattoki, district Kasur, Punjab, Pakistan. The block size was 7 m in length x 3 m in width (21 m^2) with six replications. The experiment comprised two treatments; use of inoculation and no inoculation (control).

Land preparation, fertilisers application, sowing and harvesting: The land preparation was undertaken by applying three ploughings with cultivator followed by two plankings to make a fine seedbed. Urea (46% N), di-ammonium phosphate (DAP; 18% N and 46% P_2O_5) and muriate of potash (MOP; 60% K_2O) at 20, 300 and 50 kg/ha respectively were applied to achieve the recommended application of 20, 60 and 30 kg/ha of nitrogen (N), phosphorus (P) and potassium (K) respectively. All the fertilisers were broadcast by hand and then incorporated with ploughing into the soil prior to sowing. A pre sowing irrigation was then applied to the field for wet sowing of berseem seed. The berseem clover seed was cleaned manually and inoculated with *Rhizobium trifolii* prior to sowing as described by Agarwal and Ahmad (2010). Sowing occurred during the 2nd week of October.

Three forage cuts were taken and forage harvesting was completed during the last week of March and the crop was then left for seed harvesting. The first forage cut was taken 65 days after sowing (DAS), the second was 45 days after the first cut and the third cut was taken 40 days after the second cut. The seed crop was harvested during the third week of May.

Data and sample collection: After laying out the experiment, composite soil samples were collected from different locations at random across the treatments by using a soil auger to a depth of 30 cm before (the application of *Rhizobium* inoculum and fertilisers) and after sowing and harvesting (Kandil *et al*., 2005). Soil measurements included organic matter, total N (%) and available P and K (ppm) (Estefan *et al*., 2013).

At each harvest three one m^2 quadrats were cut from within the plot and individually weighed. Plant height (cm: average of 10 plants per quadrat), and number of stems and heads per m^2 were recorded at each harvest. A composite 1.0 kg sample from each plot was oven dried (Hot Air oven/T1-OV-H-250) at 70ºC for 72h to determine dry matter (DM) content and calculate DM yield (t/ha). A second sample was dried at 60ºC for 48h prior to grinding through first a 5 mm and then a 1 mm screen, and a 150 g sub-sample of this material was then dried at 80˚C for 24h to determine neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP), DM digestibility (DMD), digestible organic matter (OM) on a DM basis (DOMD), water soluble carbohydrates (WSC) and metabolisable energy (ME) by near infrared reflectance spectroscopy (NIRS) using a Bruker multi-purpose analyser (MPA, Bruker Optik GmbH, Ettlingen, Germany) and OPUS software (version 5.1) with calibrations developed by the New South Wales, Department of Primary Industries' Feed Quality Service (FQS). The calibrations were developed using the following methods: NDF and ADF analysed sequentially (Van Soest *et al*., 1991) using the filter bag method (Ankom® 200/220 fibre analyser, ANKOM technology, Macedon, NY, USA), CP as N x 6.25 with N determined using the Dumas combustion method (Leco CNS 2000® analyser: Leco, St. Joseph, MI, USA), ash by heating a sample in a muffle furnace at 550ºC for 6h (Galyean, 2010, AFIA, 2014), DMD and DOMD by the pepsin cellulase digestibility assay (AFIA, 2014) and ME calculated as DMOD x 0.203 -3.001 (AFIA, 2014).

At the seed harvest ten seed heads were randomly selected from each plot, and the seeds removed and counted. 1000-seed weight was determined by counting out 1000 seeds and weighing and predicted seed yields (kg/ha) were calculated. In addition, data were also recorded for the number of nodules per plant. Five plants from each treatment plot were uprooted randomly. Roots were washed with clean water to remove all soil particles and then the number of nodules per plant was recorded and the average number of nodules per plant calculated (Hussain *et al*., 2002).

Statistical analysis: The data were statistically analysed with linear mixed model (ASREML) with the inoculation treatment identified as a fixed effect, and the replications and plots as random effects, by using GenStat® (17thedition) windows software (VSN International, 2014). The least significant differences (LSD) at 5% level of significance were used to compare the treatment means.

RESULTS

Forage production: Inoculation of berseem clover seed with *Rhizobium trifolii* significantly (P<0.05) increased plant growth across all plant parameters measured, as shown in

Forage **quality:** The effects of inoculation on the nutritive value of the forage is shown in

Table 2. The CP content of the forage produced using inoculated seed was significantly higher *(*P<0.05) than that produced from non-inoculated seed. Similarly, both the NDF *(*P<0.001) and WSC *(*P<0.05) contents were higher in the forage produced from inoculated seed. However, seed inoculation had no effect *(P*>0.05) on the ME, and ADF content of the forage.

Table 1. Inoculation resulted in a 16% increase (*P*<0.001) in the number of stems per m^2 and the *Rhizobium* inoculated plants were 22.7% taller (*P*<0.001) than the non-inoculated plants (

Forage **quality:** The effects of inoculation on the nutritive value of the forage is shown in

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Table 1; **Error! Reference source not found.**). In addition, inoculation increased green forage production per ha by 26.3% (*P*<0.001) and forage DM yield by 38.5% (*P*<0.05).

Forage quality: The effects of inoculation on the nutritive value of the forage is shown in

higher in the forage produced from inoculated seed. However, seed inoculation had no effect *(P*>0.05) on the ME, and ADF content of the forage.

Table **2**. The CP content of the forage produced using inoculated seed was significantly higher ($P<0.05$) than that produced from non-inoculated seed. Similarly, both the NDF ($P<0.001$) and WSC ($P<0.05$) contents were higher in the forage produced from inoculated seed. However, seed inoculation had no effect ($P>0.05$) on the ME, and ADF content of the forage.

Table 1. Effects of *Rhizobium trifolii* inoculum on forage and seed yield parameters of cv. Agaitti Berseem-2002 of berseem clover.

Treatment	Number of stems (/m^2)	Plant height (cm)	Green forage yield (t/ha)	Dry matter yield (t/ha)
Non-inoculated	300.2^a	19.9^a	31.6^a	4.0^a
Inoculated	348.2^b	24.4^b	39.9^b	5.5^b
SED	11.73	0.33	2.23	0.43

SED = standard error of differences. Values within columns with varying superscripts differ significantly ($P<0.05$).

Table 2. Effects of *Rhizobium trifolii* inoculum on forage quality parameters of cv. Agaitti Berseem-2002 of berseem clover.

Treatment	CP (%)	ME (%)	ADF (%)	NDF (%)	WSC (%)
Non-inoculated	27.6[a]	10.5	19.6	24.3[a]	1.5[a]
Inoculated	28.6[b]	10.7	21.0	26.5[b]	2.02[b]
SED	0.332	0.153	0.833	0.374	0.112

SED = standard error of differences. Values within columns with varying superscripts differ significantly (P<0.05).

Seed production: Inoculation of berseem clover seed significantly (P<0.001) improved seed production and quality across all measured seed parameters (

Table 3). The number of seed heads per m^2 increased by 43% (P<0.001) in comparison to the non-inoculated control. Additionally, inoculation resulted in a 39.4% increase (P<0.001) in the number of seeds per head and a 9.8% increase (P<0.001) in 1000-seed weight. As a consequence of the positive effects of inoculation on these parameters, the predicted seed yield was increased by 118.95% (P<0.001).

Nodulation and soil nitrogen content: The nodule count and total available soil N contents for both inoculated and non-inoculated trial plots are presented in Table 4. The number of root nodules per plant increased significantly (P<0.05) over non-inoculated plants, with a 61% increase in the nodule count. Additionally, the nodules present on inoculated roots appeared larger and distributed further along the root system on to secondary and tertiary root axes.

Table 3. Effects of *Rhizobium trifolii* inoculum on seed yield and yield parameters of cv. Agaitti Berseem-2002 of berseem clover.

Treatment	Number of heads (/m^2)	Number of seeds per head	1000-seed weight (g)	Predicted seed yield (kg/ha)
Non-inoculated	237[a]	17.6[a]	3.5[a]	146.2[a]
Inoculated	339[b]	24.5[b]	3.8[b]	320.1[b]
SED	17.79	0.505	0.071	18.21

SED = standard error of differences. Values with columns with varying superscripts differ significantly (P<0.05).

Table 4. Effects of *Rhizobium trifolii* inoculum on nodule count per plant of cv. Agaitti Berseem-2002 of berseem clover, and soil organic matter, nitrogen (N), phosphorus (P) and potassium (K) at UVAS, Pakistan.

Treatment	Number of nodules per plant	Organic matter (%)	Available		
			Total N (%)	P (ppm)	K (ppm)
Non-inoculated	63.8[a]	0.6[a]	0.02[a]	4.8[a]	140.3[a]
Inoculated	102.5[b]	0.8[b]	0.04[b]	6.1[b]	199.0[b]
SED	6.360	0.045	0.002	0.537	26.47

SED = standard error of differences. Values with columns with varying superscripts differ significantly (P<0.05).

The chemical analyses of the soil samples showed significant increases (P<0.05) in the soil OM (from 0.6 to 0.8%) and nutrients such as N (from 0.02 to 0.04%), P (from 4.8 to 6.1ppm) and K (from 140.3 to 199ppm) with the use of *Rhizobium* inoculation (Table 4). As shown in Figure **2**2, inoculation resulted in a significant increase (45.28%) in the available soil N due to an increase in the formation of root nodules (Table 4) which enhanced N fixation resulting in more available N for plant growth in the soil.

Potential impact of inoculation of berseem clover seed on the income of smallholder farmers: Inoculation of Agaitti Berseem-2002 seed resulted in increased forage and seed production. Using an average market value of 4.04 Rs/kg (PKR) for fresh forage and of 450 Rs/kg for seed (recorded in the study area), inoculation has the potential to significantly increase smallholder farm profitability. Seed inoculation produced 8 tonnes of extra forage and 174 kg seed, and resulted in an additional 111, 913 rupees of net income to farmers. The cost of *Rhizobium* inoculum was minimal (148Rs/ha) relative to the financial benefits derived from increased yields and quality of both forage and seed, and added soil N. The increase in N availability of 45% is the equivalent of one 50 kg bag of urea (containing 46% N) which saves the farmers about 2000 Rs.

Figure 1. *Rhizobium* inoculated berseem clover plants (right) grow taller and produced more leaves as compared to non-inoculated (left) plants.

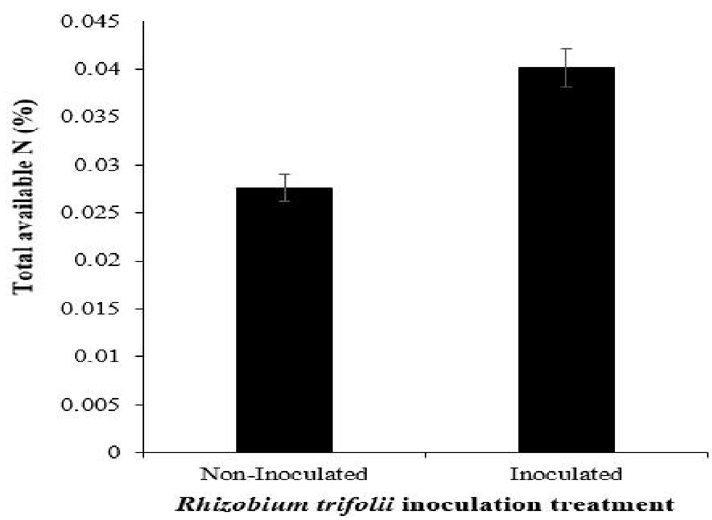

Figure 2. The graph shows the significant increase in available soil nitrogen with seed inoculation of *Rhizobium trifolii* compared to non-inoculation on cv. Agaitti Berseem-2002 of berseem clover.

DISCUSSION

Forage production: The positive effects of *Rhizobium* inoculation on overall plant growth and development of Agaitti Berseem-2002 clover plants resulted in higher forage yield. This is consistent with the results of previously reported inoculation trials (Agarwal and Ahmad, 2010, Giambalvo *et al*., 2011). The differences between inoculated and non-inoculated fresh and DM forage yields occurred as a consequence of the seed inoculation which increased nodulation and N fixation. This enhanced photosynthetic activity by leaves and increased plant growth resulting in higher green forage and DM yields, consistent with the results of Agarwal and Ahmad (2010) and Giambalvo *et al*. (2011).

The number of stems per m^2 and plant growth/height are important factors affecting forage yields. The positive effect of inoculant on both the number of stems per m^2 (16%) and plant height (22.67%) supports the findings of Hussain *et al*. (2002), Agarwal and Ahmad (2010) and Thalooth *et al*. (2015) who also demonstrated increases in relative growth rate, number of stems and fresh yield. The magnitude of the responses reported by Agarwal and Ahmad (2010) and Hussain *et al*. (2002) varied from 7 to 26% and may have varied to those in the present study (42.5 t/ha and 36 t/ha of green forage compared to 39.9 t/ha in the present study) due to the use of different cultivars responding differently to seed inoculant or perhaps the inoculants themselves (Graves *et al*., 1990).

Both fresh (green) and DM forage yields were increased by inoculation, which is similar to previously reported results (Bajpai *et al*., 1974, Hussain *et al*., 2002, Agarwal and Ahmad, 2010) who found a 74% increase in green forage yield, 20% greater green forage and 17% DM yields, and 26% increases in green forage and DM yields, respectively. This is likely due to the increased activity of the plant roots and consequently increased N fixation by nodules, resulting in the enhancement of nutrient availability and nutrient use efficiency; leading to a 21% increase in plant biomass (Thalooth *et al*., 2015). Another contributing factor would be the assimilation of higher amounts of carbohydrates in the plant because of increased photosynthetic activity (Agarwal and Ahmad, 2010) resulting from the increased number of stems or leaves, 10% increase in plant height and 13% increase in total DM. Jan *et al*. (2014) found that shoot yield increased significantly with the use of inoculation. Agarwal and Ahmad (2010) also found that the use of *Rhizobium* inoculum enhanced shoot weights by 9%, resulting in higher fresh forage yields.

The magnitude of the response to *Rhizobium* inoculation varies widely across the literature depending on the soil conditions (saline or sodic) and the cultivars used. Agarwal and Ahmad (2010) reported a 14.2% increase in DM yield (10.5 t/ha vs. 12 t/ha), whilst Jan *et al*. (2014) reported a 2.07-fold increase (2.09 t/ha vs. 4.33 t/ha) and Thalooth *et al*. (2015) a 2.6-fold increase (3.71 t/ha vs. 9.78 t/ha). Hussain *et al*. (2002) found that *Rhizobium* inoculation resulted in a 17% increase in DM yield (72.20 g/pot vs. 84.50 g/pot) compared to the 38.5% increase in DM forage yield recorded in the present study. The differences between inoculated and non-inoculated fresh and DM forage yields occurred as a consequence of seed inoculation increasing nodulation and N fixation. The majority of the nodules were found on the tap root and were of larger size in the inoculated plants compared to non-inoculated, which had smaller nodules that were located on the lateral roots. The nodules were not dissected for colour assessment; however, the larger nodule size and tap root location was assumed to be indicative of greater activity of the nodules (Hussain *et al*., 2002, Agarwal and Ahmad, 2010).

Forage quality: The inoculation of berseem clover seed with *Rhizobium* resulted in increased CP content of the forage, although it did not affect ME. This was similar to the findings of Giambalvo *et al*. (2011) who reported improved nutritive value by increasing protein levels in forage in response to *Rhizobium* inoculation. Thalooth *et al*. (2015) and Hussain *et al*. (2002) also reported increases in forage CP of 10% and 20%, respectively in response to seed inoculation. Forage CP levels have been shown to increase with access to greater supplies of soil N (Giambalvo *et al*., 2011). The increase in the CP content of the forage in response to seed inoculation may also have been a consequence of an increase in the number of leaves per plant or an increase in the leaf to stem ratio.

For milk production, NDF is important as dairy cows require sufficient NDF in their diets to maintain rumen function and maximise milk production. Increases in forage NDF can significantly enhance DM intake and NDF digestibility in dairy cows, resulting in increased milk yield. It is recommended that dairy rations contain at least 25% NDF (Oba and Allen, 1999). The forage grown from the non-inoculated seeds (24% NDF) did not meet this recommended level; however, seed inoculation resulted in a 9.19 % increase in the NDF content, which would provide sufficient NDF to dairy animals to maximise milk production. However, inoculation resulted in only a small increase in the ADF content (6.92%) of the forage. As ADF is a measure of the cellulose and lignin contents whilst NDF measures total plant cell wall material which includes hemicellulose, cellulose and lignin (Van Soest *et al*., 1991), it is likely to be of minimal importance for animal nutrition and productivity.

Seed production: The positive relationship of inoculation with plant nodule formation and forage production helps to explain the significant improvement in all of the seed yield parameters **(Table 3),** culminating in a 119% increase in seed yield. These results were in line with those of Bajpai *et al*. (1974) and Agarwal and Ahmad (2010) who also reported increased seed yields in response to inoculation using *Rhizobium* strains. The increase in seed weights was likely due to increased availability of carbohydrates for seed formation during the reproductive growth stage (Bajpai *et al*., 1974), resulting from the increased activity of the roots for efficient nutrient (N and P) uptake (Hussain *et al*., 2002, Jan *et al*., 2014).

Nodulation, soil fertility and nitrogen content: The N content of the soil increased with the growing of the berseem clover as a result of N fixation and was further enhanced (by 45% over non-inoculated treatment) as a consequence of inoculation of the seed. These results echo those of Jan *et al*. (2014), Giambalvo *et al*. (2011) and Hussain *et al*. (2002). For the non-inoculated plants, nodule counts per plant (63.8/plant) were similar (67.7/plant) to those reported by Hussain *et al*. (2002); but for the nodule counts for the inoculated plants, results were higher (102.5 /per plant) than reported by Hussain *et al*. (83.3/plant). This may have been associated with varietal differences, the inoculant, and the soils used in the two studies. Hussain *et al*. also reported a "boosting effect" of using *Rhizobium* inoculation on root growth, particularly on root dry weights (19% increase) in both non-saline and saline treatments in Pakistan. The OM, P and K contents of the soils used in the present study increased by 42%, 29% and 42%, respectively (**Error! Reference source not found.**) as a result of inoculation. The magnitude of these responses is dependent on the level and type of the *Rhizobium* present in the soil. Similar increases in OM and P contents in soil in response to seed inoculation were reported by Jan *et al*. (2014). The increased OM levels in the soil were likely associated with increased shoot and root dry weights, as reported by Jan *et al*. (2014).

The increase in dry weights of shoot and root (see **Error! Reference source not found.**) with the increased activity of *Rhizobium* in the root zone resulting in greater availability of soil nutrients to the plant (Hussain *et al*., 2002, Jan *et al*., 2014). Moreover, Jan *et al*. (2014) also reported that nutrient (P and K) uptake of berseem clover plants was increased by seed inoculation under calcareous soil conditions (pH≥8), and *Rhizobium* inoculation further increased available soil N by 41% through N-fixation (Hussain *et al*., 2002). Repeated harvesting of the crop increases utilisation of plant reserves as well as maintaining the plants in the vegetative growth stage for longer (Giambalvo *et al*., 2011). This increased nutrient (particularly N) demand in turn increases symbiotic N-fixation by *Rhizobium* present in the root nodules (Graham and Vance, 2000, Bruning and Rozema, 2013). Alternately, delaying forage cuts results in lower N-fixation due to a decline in the symbiotic N-fixation as the plant progresses to the reproductive growth stage (Giambalvo *et al*., 2011). Frequent cutting of forage at the optimum times of 65, 110 and 150 DAS is likely to have increased both yields of better quality forage and seed and the N content in the soil.

Conclusion: Both forage and seed yield components increased significantly by inoculation of cv. Agaitti Berseem-2002 berseem clover seed with *Rhizobium trifolii*. The nutritive value of the forage also increased (4% CP) due to more leaves per plant. The increased forage and seed production could potentially generate an additional net income of PKR 111,913 Rs/ha (US$ 1145/ha) and increase the profitability of the crops grown subsequently in the same field. Therefore, the practice of inoculating seed prior to sowing berseem clover crops in Pakistan offers a cheap and easy method of increasing the productive potential of the forage crop and therefore the profitability of the low input farming systems in which berseem clover dominates winter forage production. Further studies into the use of seed inoculation, removal of basal N applications and its effects on growth and farm economics as well as barriers to adoption by small-holder farmers now need to be considered to determine why this readily available technology has not been utilised to date.

Acknowledgements: We are thankful to the Australian Centre for International Agricultural Research (ACIAR) for providing the financial support to undertake this research. We are grateful to the School of Animal and Veterinary Sciences, Charles Sturt University for awarding the Faculty of Science Scholarship to the senior author. We also thank the University of Veterinary and Animal Sciences, Lahore for supporting and providing the facilities to undertake the research work.

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