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Effect of Different *Rhizobium* Inoculation Methods on Performance of Lentil in Pothowar Region

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ABSTRACT

To determine the effect of different *Rhizobium* inoculation methods on yield, yield components, root nodulation, seed protein contents and N-contents of plant and soil, investigations were done in pot experiment under natural conditions at University of Arid Agriculture, Rawalpindi, during fall 2004. The research material tested consisted of two lentil varieties i.e. Masoor-93 and Massor-2002, while treatments of seed inoculation, soil inoculation and both seed + soil inoculation were compared against control (no inoculation). The variables such as seed protein contents, root nodulation, N-contents of shoot and root of lentil, N-contents of soil, seed yield and yield components were significantly affected by *Rhizobium* inoculation. Inoculation of seed and soil was found more effective in case of seed yield, protein contents of seed and dry weight of nodules plant^{-1}. The variety Masoor-2002 performed better in all variables except protein contents when compared with Masoor-93 and thus resulted higher yield. The variety Masoor-2002 gave maximum seed yield with only *Rhizobium* seed inoculation, while the variety Masoor-93 produced maximum seed yield, when seed and soil both were inoculated with *Rhizobium.*

Key Words: Yield; Nodulation; N-content; *Rhizobium* inoculation; Protein content; *Lens culinaris*

INTRODUCTION

Lentil (*Lens culinaris* Medic) is an important rabi crop in Pakistan and is usually grown on rainfed areas. The area sown under this crop during the year 2005-06 was 33.9 thousands hectares in the country with total production of 17.9 thousands tones, resulting an average yield of 528 kg ha^{-1} (Government of Pakistan, 2005-06). The yield of pulses in Pakistan is lower than many other Asian countries. The reasons of low yield of pulses especially in Pothowar are numerous but a nutritional imbalance appears to be the major one. In general, soils of rainfed areas of Pakistan are deficient in plant nutrients and have very low organic matter contents (Ahmad *et al*., 1988), which often found to be less than even one percent under Pothowar conditions.

Traditional use of chemical fertilizers in agriculture production can not be over emphasized. The cost of chemical fertilizers is becoming un-affordable for the farmers of rainfed areas in addition to creating soil and water hazards. There is great need to supplement or substitute chemical fertilizers with organic manures or to explore biological means to improve the soil health. The organic manures are not available in the Pothowar region and what ever is available, the people use it as fire wood rather than to use it for soil fertility. Therefore, there is need to explore the potential of *Rhizobia* as legume inoculants under agro climatic conditions of the Pothowar region.

Lentil, like other legumes, has the ability to fix atmospheric nitrogen through symbiotic association with *Rhizobia*. The ability of symbiotic fixation may offer an opportunity to improve nitrogen status of the soil and crop productivity under rainfed conditions. More than 85% of the required nitrogen needs of lentil can be met by symbiotic nitrogen fixation through inoculation with effective r*hizobium* strains in the field thus yield could be increased up to 2 ton ha^{-1} (Bisen *et al.*, 1980). Many researchers carried out experiments on *rhizobium* inoculation with and without fertilizers on mungbean crop (Malik *et al*., 2002; Ashraf, 2003; Hayat *et al.*, 2004; Muhammad *et al.*, 2004; Nadeem *et al.*, 2004) and found increased nitrogen contents of seed, number of nodules, yield and yield components.

Singh and Tarafdar (2001), Hoque and Haq (1994) and Khurana *et al*. (1995) have recorded significantly increased yield of lentil by inoculating with *Rhizobium* compared with uninoculated plants receiving no nitrogen fertilizer. Although lots of studies are already being conducted else where on the subject but the soil is diversified and dynamic nature. In rapidly changing climatic conditions it is imperative to study the effectiveness of r*hizobia* in fixing atmospheric N and its subsequent effect on legume crop in a given soil. Therefore, a new study on scientific lines on the *Rhizobium* inoculation to generate latest information in order to reduce the costly inputs of fertilizers may prove helpful for the farming community of Pothowar.

In view of the above facts, a study was conducted with objective to compare different *Rhizobium* inoculation methods on the performance of lentil crop under rainfed conditions of Pothowar.

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MATERIALS AND METHODS

To evaluate effects of *Rhizobium* inoculation methods on performance of two selected cultivars of lentil, a pot experiment was conducted under natural environment at University of Arid Agriculture, Rawalpindi during the year 2003-2004. The soil used in experiment was sandy loam containing total nitrogen 0.045%, nitrates 3 ppm, potassium 60 ppm and phosphorus 4.5 ppm. The experiment was laid out in completely randomized design with three replications. Two lentil genotypes viz Masoor-93 and Masoor-2002 were sown in earthen pots having 30 cm height with 30 cm top and 15 cm bottom diameter. Each pot was filled with 5 kg dry soil having 50:50 sand clay mixtures. For seed inoculation, seeds were coated with paste of inoculums containing 10^7 *Rhizobium leguminosarum* per gram. Ten (10) seeds were sown in each pot. For soil inoculation one liter solution of *Rhizobial* inoculum containing same number of *Rhizobia* was added to the soil in each pot before sowing. In the third treatment both seed and soil were inoculated keeping *rhizobial* constant. All other management practices were kept uniform for all the treatments. Data on root nodulation, seed protein content, N-content of root and shoot of lentil were recorded at initiation of flowering where as yield and yield components were taken at maturity. The N-contents in seeds were determined by Micro Kjeldahl Method according to the procedure given by the Association of Official Agriculture Chemist (A.O.A.C., 1990). Protein contents were calculated by multiplying the N-contents by the factor 5.70. For root nodulation five plants from each pot were uprooted and their nodules were counted and then averaged. The soil N-contents were determined after harvesting using total nitrogen method (Jackson, 1962). For N contents of roots and shoots five plants were selected from each pot at flowering stage. The nodules were removed and dried in the oven. These samples were ground and sieved. The total nitrogen contents of roots, shoots and nodules were measured by total nitrogen method (Jackson, 1962). The data recorded were subjected to statistical analysis using Fisher's analysis of variance techniques. Least significant difference test at 5% probability level was used to compare the differences among treatment means (James *et al*., 1997).

RESULTS

Soil N-content. N-content of soil is an important parameter for determining the nitrogen fixing ability of plants. The results pertaining to soil N content after harvest showed significant interaction between all the treatments and varieties (Table I). Soil N-content increased due to *Rhizobium* inoculation. The investigation showed that all inoculated treatments significantly improved the soil N contents after harvesting of the crop except seed inoculation for Masoor-2002. Maximum soil N content (0.41%) were recorded in case of seed + soil inoculation closely followed by soil inoculation alone in Masoor-2002, while minimum soil N contents were observed in seed inoculation in case of Masoor-93 closely associated with control treatment of the same variety. This major change among treatments might be due to application of soil and seed inoculum used in the experiment. N-content of soil after harvesting increased as compared with N-content of soil before sowing by *rhizobial* inoculation.

N-content of root. Data regarding N-content of root revealed that inoculation methods and interaction between varieties and inoculation method were highly significant (Table II). Soil inoculation significantly increased the N-content of root in both genotypes of lentil as compared to control. The lowest N-content of roots were recorded in case of Masoor-93. The N-contents of roots of Masoor-2002 was statistically similar in all the three methods of inoculation, while Masoor-93 showed higher root N-contents in response to soil inoculation as compared with two other methods evaluated in the experiment.

N-content of shoot. The data regarding the varieties, the inoculation methods and shoot N- content revealed that the interaction between the inoculation treatments and the varieties were significant (Table III). Between varieties, the shoots of Masoor–2002 exhibited significantly higher N-contents than Masoor-93. Soil inoculation although showed maximum N-contents of shoot in both the varieties but was at par with the combination of both the methods. Application of inoculation showed better results and was more effective as compared to control. The lowest shoots N content were recorded in the shoots of Masoor-93 in control.

Seed protein contents. Seed protein contents revealed that the effect of main treatments and interaction between methods of inoculation and varieties were highly significant (Table IV). In case of Massor-93 seed inoculation alone and in combination with in soil inoculation increased significantly protein content as compared to sole soil inoculation, where as for Masoor-2002 the difference among three methods remain non-significant though the seed inoculation and soil inoculation in combination increased seed protein contents against control. However, both the varieties did not differ for their seed protein contents.

Number of pods plant^{-1}. There was no significant difference among main treatments i.e., varieties and methods of inoculation but the interaction between varieties and inoculation methods were significant (Table V). Masoor-2002 produced highest pods plant^{-1} in case of seed + soil inoculation, which was at par with soil inoculation alone. Number of pods plant^{-1} in Masoor-93 significantly increased by soil inoculation alone and in combination with seed inoculations compared to control. Masoor-93 with soil inoculation and Masoor-2002 with seed inoculation showed statistically same number of pods plant^{-1}, while in rest of the treatments Masoor-2002 produced more pods than Masoor-93.

Test weight. Both the varieties showed significant difference for 100-seed weight and interaction between varieties and method of *Rhizobium* inoculation was also

Table I. Soil N-content (%) under lentil crop as affected by *Rhizobium* inoculation methods

Treatments	Masoor-93	Masoor-2002	Mean
Control	0.30f	0.38bc	0.34B
Seed inoculation	0.28g	0.37cd	0.32C
Soil inoculation	0.36d	0.39ab	0.37A
Seed +Soil inoculation	0.32e	0.41a	0.36A
Mean	0.31B	0.38A	

LSD (0.05) for verities = 0.0122
LSD (0.05) for treatments = 0.0099
LSD (0.05) for interaction (Varieties + Treatments) = 0.0173

Table II. N-content of lentil root (%) as affected by *Rhizobium* inoculation methods

Treatments	Masoor-93	Masoor-2002	Mean
Control	2.65b	2.70b	2.69 C
Seed inoculation	2.69b	2.82a	2.74 B
Soil inoculation	2.83a	2.85a	2.84 A
Seed +Soil inoculation	2.65b	2.82ab	2.73 B
Mean	2.70B	2.79A	

LSD (0.05) for treatments = 0.07
LSD (0.05) for varieties = 0.05
LSD (0.05) for interaction (Varieties = Treatments) = 0.547

Table III. N-content of lentil shoots (%) as affected by *Rhizobium* inoculation methods

Treatments	Masoor-93	Masoor-2002	Mean
Control	2.55e	2.78c	2.66C
Seed inoculation	2.61f	2.80bc	2.70B
Soil inoculation	2.70d	2.88a	2.77A
Seed + Soil inoculation	2.70d	2.84ab	2.77A
Mean	2.64B	2.82A	

LSD (0.05) for treatments = 0.0387
LSD (0.05) for interaction varieties =0.10
LSD (0.05) for interaction (varieties treatment) = 0.0547

Table IV. Protein content (%) of lentil seed as affected by *Rhizobium* inoculation methods

Treatments	Masoor-93	Masoor-2002	Mean
Control	22.54a	18.50b	20.52B
Seed inoculation	21.54a	21.53a	21.53A
Soil inoculation	17.86b	21.51a	19.68C
Seed+Soil inoculation	21.51a	21.53a	21.55A
Mean	20.86NS	20.77NS	

LSD (0.05) for treatment =0.7818
LSD (0.05) for interaction (Varieties x Treatments) =1.106
*Non-Significant

significant however, no significant difference recorded between methods of inoculation (Table VI). Soil inoculation treatment produced maximum 100-seed weight in both the genotypes of lentil followed by seed + soil inoculation treatment.

Seed yield per plant. There was significant effect of both main treatments and their interactions (Table VII). Among the methods of inoculation, seed + soil *Rhizobium* inoculation performed better than their individual effect followed by seed inoculation. In case of varieties, the variety Masoor-2002 produced significantly higher yield (2.46 g) than its counter part variety Masoor-93, which resulted average yield (1.78 g plant^{-1}).

Nodules dry weight per plant. Data regarding nodules dry weight plant^{-1} showed significant interaction between inoculation treatments and the varieties (Table VIII). The variety Masoor-2002 produced maximum nodule dry

Table V. Effect of different *Rhizobium* inoculation methods on number of pods per plant of lentil

Treatments	Masoor-93	Masoor-2002	Mean
Control	11.68e	12.34b	12.01NS
Seed inoculation	11.79de	12.10c	11.94
Soil inoculation	12.09c	12.50a	12.29
Seed + Soil inoculation	11.86d	12.60a	12.23
Mean	11.85NS	12.38NS	

LSD (0.05) for interaction (varieties x treatments) = 0.1224
NS = Non-significant

Table VI. Effect of different *Rhizobium* inoculation methods on 100 seed weight (g) of lentil

Treatments	Masoor-93	Masoor-2002	Mean
Control	2.95d	3.45b	3.20NS
Seed inoculation	3.03d	3.55b	3.29
Soil inoculation	3.45b	3.95a	3.70
Seed + Soil inoculation	3.30c	3.85a	3.57
Mean	3.18B	3.70A	

LSD (0.05) for varieties = 0.547
LSD (0.05) for interaction (varieties x treatments) = 0.1095
NS = Non-significant

Table VII. Effect of different *Rhizobium* inoculation methods on seed yield (g plant^{-1}) of lentil

Treatments	Masoor-93	Masoor-2002	Mean
Control	1.66de	2.13c	1.89BC
Seed inoculation	1.30e	3.10a	2.20B
Soil inoculation	1.36e	2.10cd	1.73C
Seed + Soil inoculation	2.80ab	2.53bc	2.66A
Mean	1.78B	2.46A	

LSD (0.05) for varieties = 0.2240
LSD (0.05) for treatments = 0.3168
LSD (0.05) for interaction (varieties x treatments) = 0.4480

Table VIII. Effect of different *Rhizobium* inoculation methods on nodule dry weight per plant of lentil

Treatments	Masoor-93	Masoor-2002	Mean
Control	1.58g	1.76d	1.67D
Seed inoculation	1.57h	1.79c	1.68C
Soil inoculation	1.68f	1.84a	1.71B
Seed + Soil inoculation	1.74e	1.82b	1.78A
Mean	1.64B	1.80A	

LSD (0.05) for varieties = 0.10
LSD (0.05) for treatments = 0.0038
LSD (0.05) for interaction (varieties x treatments) = 0.0054

weight in response to soil inoculation followed by combination of seed and soil treatment. Almost, similar trend was exhibited by variety Masoor-93 in relation to methods of inoculation. However, seed inoculation treatment in case of variety Masoor-93 produced lowest nodule dry weight followed by soil inoculation and then by combination of seed and soil inoculation.

DISCUSSION

The data recorded suggested that inoculating seed only was less effective as indication of bacterial survival started decreasing soon after seed inoculation. However, soil inoculation can compensate for it as the chances of *rhizobial* mortality in soil were reduced resulting more effective population of bacteria to induce more nodules and their dry weight. The results also revealed that N-content of soil analysed after harvesting increased as compared with N-content of soil before sowing. Rashid *et al*. (1999) also

attributed it to symbiotic fixation of atmospheric nitrogen by inoculated strain of *rhizobium*. The nitrogen fixed in plant nodules is partly used by the plant as indicated by rise in N contents of plant root and shoot. The remaining nitrogen is added to the soil and can be utilized by the subsequent crop. Similar kinds of observations were reported by Daterao *et al.* (1990) who recorded increased soil nitrogen after the harvest of green gram. The increase in nitrogen uptake by plant due to inoculation application was also reported by Basu and Bansyopadhyay (1990) and Rashid *et al.* (1999).This increased uptake of nitrogen by plants led to increased seed protein contents as Kantar *et al.* (1994) and Hoque and Haq (1994) also reported that *Rhizobium* inoculation significantly affected seed protein content. It seems that the higher nodule dry weight, root and shoot N contents supplied sufficient N for synthesis of amino acids (the building block of protein) that translated in higher seed protein contents. The increase in seed protein percentage can be linked with better N-supply to the plant.

Simply inoculating seed did not significantly affect 100-seed weight of both the varieties against control. This limits that increased N supply did not increase seed filling phase of reproductive growth. Nevertheless, the quality of seed improved in terms of protein contents. Both the varieties responded significantly to *Rhizobium* inoculation however, the variety Masoor-2002 exhibited higher seed weight indicating its genetic potential to direct the assimilates efficiently towards sink. Similar results were reported by Hoque and Hoq (1994) and Nadeem *et al.* (2004) who found that number of pods plant^{-1} was significantly affected by seed inoculation. These results were in agreement with the result of Asghar *et al.* (1988), Kumar *et al*. (1993) and Namdeo *et al.* (1996). Both the varieties responded positively to seed and soil inoculation. However, the variety Masoor-2002 out yielded Masoor-93 even with seed treatment alone compared to the rest of treatments clearly showing better yield potential. Sekhon *et al*. (2002) recorded that *Rhizobium* inoculation resulted higher seed yield over no inoculation.

CONCLUSION

Inoculation of soil and seed with *Rhizobium* significantly influenced seed protein content, N-content of shoots and roots of lentil and soil, which consequently led to improved soil fertility and can reduce the production cost of next crop through reduced input in the form of nitrogen fertilizers, which in turn also minimize the health hazard effects. *Rhizobial* inoculation was found to be more effective and produced better yield through positive effect on yield components when compared with no inoculation. However, applying inoculum to both seed and soil simultaneously proved more effective. Masoor-2002 had larger seeds thus yielded better than Masoor-93 but there was no significant difference in seed protein contents of both the varieties. More field trial are imperative in the agro-ecological regions of Pothowar to confirm these findings.

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