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Rhizobial Inoculation Techniques with Different Nitrogen Fractions Improved Productivity of Maize-Soybean Intercropping System

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Abstract

In a cereal legume intercropping, leguminous crops always demand the application of appropriate inoculants which ultimately improves efficiency of applied fertilizer and yield of companion crop. Rhizobium is a group of bacteria capable of providing nutrients for soybean crops. When symbiotic with legume crops, this group of bacteria is able to infect plant roots and form root nodules. A field trial was laid out to evaluate the effect of rhizobia inoculation techniques and nitrogen rates on the production potential of soybean and maize, intercropped in autumn season at Agronomic Research Area, University of Agriculture, Faisalabad. The experiment was carried out in randomized complete block design (RCBD) with factorial arrangement having three replications. Factor A was nitrogen (N) rates i.e., 0%, 50% and 100% of the recommended dose of N (250 kg ha^{-1}) for maize and factor B was inoculation techniques i.e., no inoculation, soil inoculation and seed inoculation. Results indicated that both factors not only improved 100-seed weight, seed oil contents and protein contents of soybean but also plant height, 1000-grain weight and biological yield of maize. Interaction showed that 100% N along with seed inoculation not only enhanced number pods per plant (27.00) and grain yield (1.25 t ha^{-1}) of soybean but also number of grains per cob (325) and grain yield (5.75 t ha^{-1}) of maize. However, a notable decline in grain yield of maize (2.19 t ha^{-1}) and soybean (0. 50 t ha^{-1}) were observed in those plots where application of nitrogen and inoculants were not carried out. It was concluded that in maize-soybean intercropping, 100% N for maize and soybean seed inoculation were the best strategy to enhance the yield of both crops on sustainable basis.

Keywords: Inoculation techniques, Nitrogen, Intercropping, Maize, Soybean

Introduction

Pakistan is not self-adequate in edible oil production that is why its large quantity is being imported. The native production is only 0.446 million tons while the oil seed import bill is Rs.152.541 billion (July 2016-March 2017). Continuously rising population pressure increases the per unit oil consumption (Ahmad, 2014). There is need to increase the oil seed refined area. There is no space in the traditional fixed cropping systems for oil seed crops. Therefore, research struggles should be made to improve local oilseed production so that import bill could be reduced. Due to high oil contents, soybean has potential to narrow down the difference between edible oil's consumption and production. So, intercropping can be viable answer of above said miserable situation. Its adjustment in the cropping system is very difficult due to its overlying growing season with spring and autumn maize. Moreover, farmers of maize belt do not compromise on maize for soybean cultivation. In intercropping, two or more crops in the same field are collectively planted (Lithourgidis et al, 2011). Growing of two or more crops at the same time can increase the productivity per unit of land (Woolley and Davis, 1991). The production is additional and can be attributed to the higher growth rate and decreasing the weeds. Intercropping could be a beneficial technique especially for small scale farmers (Muneer et al, 2004).

Maize-soybean intercropping system is the best approach for increasing the oilseed production because maize is a cereal crop and it accepts short duration legumes for intercropping (Maluleke et al, 2005). In intercropping, the benefit cost ratio (BCR) enhances while the component crop yield remains low because multiple cropping increases the yield by competent consumption of resources (Banik et al, 2006).

Soybean as a leguminous crop, reserves the surplus N in soil by symbiotic N_2 fixation (Bergersen et al, 1985). In legumes a key biological process is symbiotic nitrogen fixation which maintenance plant growth and the yield of high-protein seed and forage (Peoples et al, 2009). The effectiveness of biological nitrogen fixation (BNF) relies on rhizobial strain, variety and type of crop, interaction between plant varieties and bacterial strains, environmental issues and management practices. Soybean inoculation with good quality rhizobium inoculants can help marginal farmers to improve their yield and farm income (Zarrin Fatima, 2007). Rhizobial bacteria develop nitrogen fixing nodules by contaminating the roots of soybean and play a significant role in nitrogen cycle. These bacteria not only fix large amount of nitrogen which is transferred to plants, but also reduce the demand of nitrogenous fertilizer (Prakamhang et al, 2015). Rhizobial inoculants may also bring an increase in number of root hairs and lateral roots thereby favoring nutrient uptake by assessment of a greater soil volume (Biswas et al, 2000). There is possibility to increase the production of soybean by inoculating soil or seed. In maize-based cropping system, soybean inoculated with rhizobium can be helpful in supplying nutrients for sustaining soil fertility, especially nitrogen which is a critical nutrient in maize production.

It is assumed that the maize soybean intercropping with different N rates and inoculation techniques would be a best strategy to adjust soybean in existing cropping systems with the improved productivity of both crops.

Materials and methods

Experiment and treatment description

Planned experiment was laid out on sandy clay loam soil at Agronomy farm, University of Agriculture Faisalabad (Pakistan) in autumn, 2018. The experimental site was fertile with fine texture and uniform soil particles. Maize was sown by using recommended production technology except nitrogen in each experimental plot while soybean was intercropped with maize by using different set of treatments. This field trial was carried out by using randomized complete block design (RCBD) with factorial arrangements with following treatments, Factor A: Nitrogen rates **(**N_1: 0%, N_2: 50% and N_3: 100% of the recommended dose of N) Factor B: Rhizobial inoculation methods (I_1: No inoculation, I_2: Soil inoculation I_3: Seed inoculation). Sowing of both crops was done on July 24. The experiment was replicated three times with net plot size of 2.4 m × 5 m. for all treatments in maize soybean intercropping

Crop husbandry

Maize and soybean was intercropped by using 90 cm apart double row maize plantation by making beds. On each bed, two rows of both crops were maintained. The seedbed was prepared by 2-3 ploughing followed by planking. Sowing was done on beds by traditional chopa method and hand drill by using 25 kg ha^{-1} and 75 kg ha^{-1} seed rate for maize and soybean respectively. Maize hybrid, **PBG-ProA** and soybean variety, **Faisal Soybean** were used for the experiment. Urea, SSP and SOP were used as a source for N, P and K individually and no extra fertilizer was added for soybean. Other than N, both P and K were applied at; 125 kg ha^{-1} using single super phosphate (SSP) and sulphate of potash (SOP). Complete P, K and half of N was applied using urea as a source for N at sowing whereas other half N was added with first irrigation.

Weeds were controlled using Voltrel 63Se (Atrazine + S- Metalacholor + Mezotrion) as pre-emergence dose. This is a herbicide from Avoul group for pre-emergence weed control in soybean and maize. Voltrel controls certain grasses and broadleaf weeds in soybean and maize. The application rate of this herbicide is 1000 ml/acre. Moisture is necessary to activate this herbicide in soil for residual weed control so, voltrel was applied after the seed sowing of maize and soybean when field is moist.

Conquest 24% EC (Lactofen) was applied as post-emergence. This is a herbicide from Kanzo AG for post-emergence weed control in soybean and maize. Conquest controsl all types of weeds in soybean and maize including broadleaf weeds without the harmful effect on soybean crop. The application rate of this herbicide is 300 ml/acre.

Observations

Yield and Yield Components of maize

Ten cobs were taken from each experimental plot and total grains were count up and average was worked out. After that these grains were weighed to determine the 1000-grain weight. Crop from each plot was harvested at maturity and sun dried separately. The stalks along with cobs were weighed on digital balance to determine biological yield which was converted into t ha^{-1}. Grain yield of each plot was recorded after shelling the cobs following Mehboob et al (2018).

Yield and Yield Components of soybean

Plant height of ten randomly selected plants was taken with the help of measuring tape and then averaged while their number of pods were counted and averaged. Crop from each plot was harvested at maturity and sun dried separately, after those grains were weighted to determine 1000 grains weight. The stalks along with pods were weighed on digital balance to determine biological yield which was converted into t ha^{-1}. All the grains were weighted and converted into kg/ha to determine grain yield.

Grain protein content (%)

Nitrogen contents of collected grain were measured through the micro-Kjeldhal method (Anonymous, 1990) and multiplied by 6.25 to record protein contents

Grain oil content (%)

Soxhlet method described by Low (1990) was used to determine oil contents

Statistical analysis

Collected data was statistically analyzed by variance technique and difference amid treatments was compared by HSD (Honest Significant Differences) test at 5% probability (Steel *et al*., 1997).

Results

Maize

Regarding various nitrogen rates, maximum plant height, 1000-grain weight and biological yield (196.68 cm, 275.53 g and 17.15 t ha^{-1} respectively) of maize were obtained with N_3 (100% of the recommended dose of N) (Table 1) while minimum their minimum values (135.29 cm, 217.39 g and 12.37 t ha^{-1}respectively) were obtained with N_1 (0% N) in maize soybean intercropping (Table 1). Similarly in case of inoculation techniques, maximum plant height, 1000-grain weight and biological yield of maize (173.30 cm, 255.58 g and 15.48 t ha^{-1} respectively) were noted in with seed inoculation treatment (I_3) was done while their minimum values (157.68 cm, 241.12 g and 14.93 t ha^{-1} respectively) were obtained with N_1 (no inoculation) (Table 1). In case of interaction of both factors (nitrogen rates and inoculation techniques), maximum no. of grains per cob and grain yield of maize (325.00 and 5.75 t ha^{-1}) were obtained with N_3I_3 (100% of the recommended dose of N with seed inoculation) (Fig. 1 and Fig. 2). While their minimum values (211.00 and 2.19 t ha^{-1}) were noted with N_1I_1 (0% of the recommended dose of N with no inoculation) (Fig. 1 and Fig. 2).

Soybean

Regarding various nitrogen rates, maximum plant height, 1000- seed weight, seed oil and protein content of soybean (77.64 cm, 129.58 g, 18.44 % and 37.15 %) were obtained with N_3 (Table 2) while their minimum values (46.63 cm, 101.42 g, 12.21 % and 19.34 %) were obtained with N_1 (0% of the recommended dose of N) (Table 2). regarding inoculation techniques, maximum plant height, 1000- seed weight, seed oil and protein content of soybean (65.87 cm, 120.08 g, 16.50 % and 29.87 %) were obtained with I_3 (seed inoculation) while their minimum values (58.23 cm, 111.45 g, 14.58 % and 26.97 %) were noted with I_1 (no inoculation) (Table 2). However, interaction of both factors showed that maximum no. of pods per plant, biological and grain yield of soybean (27.00, 2.67 t ha^{-1} and 1.25 t ha^{-1} respectively) were noted by N_3I_3 while their minimum values (13.33, 1.53 t ha^{-1} and 0.50 t ha^{-1} respectively) were noted with N_1I_1 (Fig. 3, Fig. 4 and Fig. 5).

Economic analysis

The economic analysis of the experiment was done to look into experimental results from farmer̀s point of view as they are mainly interested in benefits and cost of a certain technology and also they like to know about risks involved in the adoption of new practices.

The efficiency of an intercropping system is determined either by the net income per unit area in a specified period of time, or benefit cost ratio (BCR). Data regarding economic analysis are presented in Table 3. In this analysis, prices of inputs prevailing in the local market were used to calculate the economic analysis of different intercropping systems. A glance at the Table indicated that maize + soybean variety (Faisal soybean) 100% recommended dose of N with seed inoculation gave the highest net income of Rs. 73084.85 per hectare.

In term of benefit cost ratio (BCR) as shown in Table 3, the highest value (1.40) was recorded in case of maize + soybean variety (Faisal soybean) 100% of the recommended dose of N with seed inoculation. The minimum BCR value of 0.62 was recorded when maize was grown with soybean variety (Faisal) 0% of the recommended dose of N with no inoculation. These results are in line with those reported by Nazir *et al*. (2000) who stated that all intercropping systems gave substantially more net income than pure stand.

Discussion

Maize

Results revealed that different inoculation techniques and different N application levels significantly affected maize productivity. A plant height is an important agronomic parameter which indicates crop plant growth. Nitrogen being a major food for crops is integral constituent of proteins (build from amino acid) and chlorophyll impart dark green color, promote leaves, plays important role in function of plant resulting in taller plants then control help in increasing the stem length which result in higher biological yield (Bloom, 2015; Hemerly, 2016). Higher grain yield was due to improved no. of grains and their weight with the application of N and inoculation of soybean (Table 1) which are key yield attributes of maize (Shah et al, 2003). Legume crops have high N fixation potential (Latt et al, 2009) which improved nutrient cycling. Improved microbial activity through inoculation enhanced the N uptake and other minerals and reduced the rate of chemical fertilizers (Latt et al, 2009) for other crop in intercropping. Furthermore, Legume decreases the nitrate (NO^{-3}) leaching risk and reduces synthetic N fertilizers requirement for other crop (Singh and Kaur, 2012; Nawaz et al, 2017). Results of this experiment are constant with that of Yang et al (2014) and Wang et al (2017) who revealed that intercropping improved resource utilization, land productivity and nitrogen availability by inoculation of legume crop and are also similar with statement that N supply improve crop yield (Yang et al, 2013). Furthermore, cereal and grown together in intercropping improves soil N and reduces artificial N requirement (Belcher and Keddy, 1995; Xiao et al, 2004; Lv et al, 2014). Li et al (2001) has also reported that combine addition of inorganic N coupled with atmospheric N fixed by other legume crop reduce the N competition in intercropping and increase the yield of both crops.

Soybean

Improved yield and yielding attributes of soybean with inoculation might be result of high association between inoculation and different nitrogen rates. Higher plant height and yield of could be credited to high N fixation by seed inoculated legume crop which increased availability of N. The seed inoculation of soybean produced greater plant height than on soil inoculation (Alam et al, 2015; Janagard and EbadiSegherloo, 2016). Pod number and grain weight increased with nitrogen levels and seed inoculation attributed to the increased N supply and its uptake in soil due to the nitrogen fixation (Janagard and Ebadi-Segherbo, 2016). Combined application of N and seed inoculation had maximum effectiveness on soybean growth (ElShaarawi et al, 2011). Better N supply throughout the growing season could be another reason of such results. Adequate addition of N supplemented with nitrogen fixation improves the flowering, grain development and proper seed setting (Shah et al, 2003). N absorbed by the plants is responsible for fixation of carbon skeleton to amino acid synthesis and it results in several proteins that have specific function in plant metabolism (Marschner, 1995). These stored amino acids and proteins result in positive increase in grain weight (Marschner, 1995; Okumura et al, 2011). Improved grain yield is attributed to sufficient nitrogen supply throughout the growing seasons by nitrogen fixation and artificial fertilizer application. The results are supported by Ullah (2015) and Matusso (2013) who concluded that grain yield and other parameters were considerably improved by inoculation techniques and increasing nitrogen rates in maize-soybean intercropping.

With regard to protein content, there was an increase of 9.7% in protein contents by seed inoculation as compared with no inoculation (Table 2). Similarly, Tahir et al (2009) and Egamberdiyeva, Qarshieva, and Davranov (2004) has reported 9% and 23% improved protein content of soybean by seed inoculation with rhizobia strain compared to no inoculation. Furthermore, N availability during seed filling stage leads to high seed protein content. In this study, maximum protein contents were observed with 100% nitrogen. Peak et al (1997) also concluded improved protein contents of seed with increasing N. Volman et al (2000), also revealed that protein contents of seed were significantly improved by N fertilization at flowering stage and rhizobium seed inoculation compared with to control.

Conclusion

It was concluded that in maize-soybean intercropping, application of 100% recommended dose of N for maize (250 kg ha^{-1}) and soybean seed inoculation were the best strategy to enhance the yield of both crops on sustainable basis.

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Table 1: Agronomic and yield related traits of autumn maize as influenced by different inoculation techniques and nitrogen rates

	Plant height	**1000 grain weight**	**No. of grains per cob**	**Biological yield (t ha^{-1})**	**Grain yield (t ha^{-1})**
Nitrogen rates					
N_1:0% of the recommended dose of N	135.29 C	217.39 C	214.78	12.37 C	2.26
N_2:50% of the recommended dose of N	164.08 B	251.54 B	270.33	16.13 B	4.29
N_3:100% of the recommended dose of N	196.68 A	275.53 A	313.44	17.15 A	5.36
HSD ($\leq$ 0.05)	7.09	13.91	9.30	0.44	0.19
Inoculation techniques					
No inoculation (I_1)	157.68 C	241.12 B	254.22	14.93 B	3.73
Soil inoculation (I_2)	165.07 B	247.77 AB	266.44	15.24 AB	3.99
Seed inoculation (I_3)	173.30 A	255.58 A	277.89	15.48 A	4.20
HSD ($\leq$ 0.05)	7.09	13.91	9.30	0.44	0.19
Interaction (N × I)	NS	NS	Fig. 1*	NS	Fig. 2*
HSD ($\leq$ 0.05)			22.22		0.46

Means sharing different letters within a column differ from each other at $p \leq 0.05$
NS = non-significant; * = Significant at $p \leq 0.05$

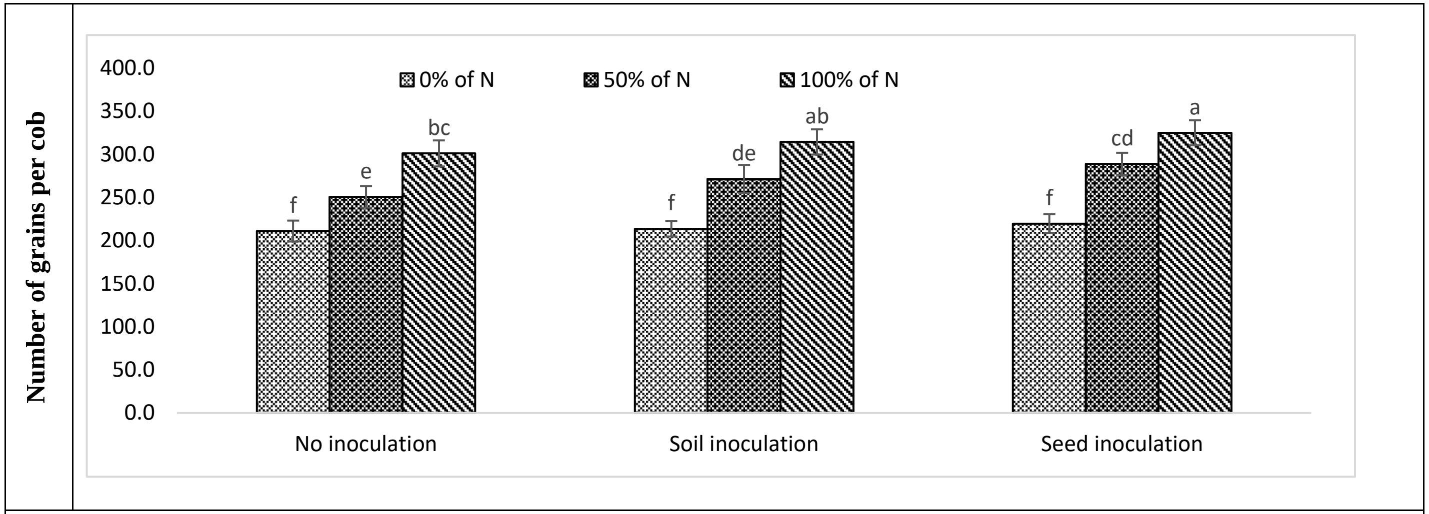

Fig. 1: Interactive effect of nitrogen rates and inoculation techniques on number of grains per cob of maize.

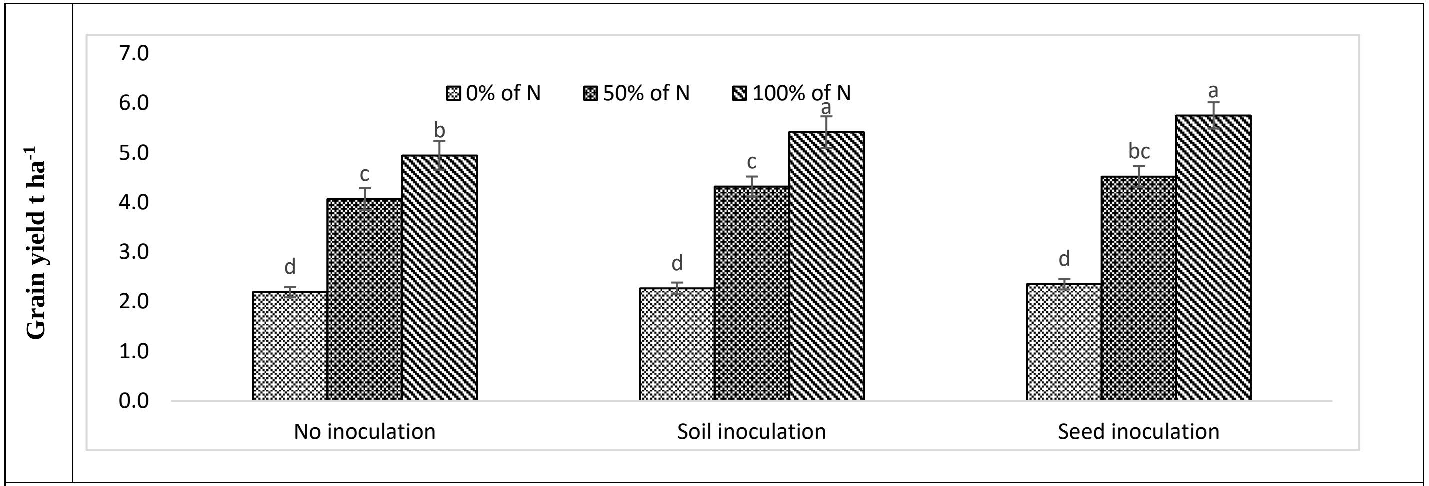

Fig. 2: Interactive effect of nitrogen rates and inoculation techniques on grain yield t ha^{-1} of maize.

Table 2: Agronomic and yield related traits of autumn soybean as influenced by different inoculation techniques and nitrogen rates

	Plant height (cm)	**No. of pods per plant**	**1000 Seed weight (g)**	**Biological yield (t ha^{-1})**	**Grain yield (t ha^{-1})**	**Oil contents (%)**	**Protein contents (%)**
Nitrogen rates							
N_1:0% of the recommended dose of N	46.63 C	13.66	101.42 C	1.60	0.55	12.21 C	19.34 C
N_2:50% of the recommended dose of N	61.70 B	18.33	116.12 B	1.94	0.87	16.16 B	29.04 B
N_3:100% of the recommended dose of N	77.64 A	24.88	129.58 A	2.50	1.15	18.44 A	37.15 A
HSD (≤ 0.05)	2.98	1.58	7.35	0.04	0.02	0.83	0.80
Inoculation techniques							
No inoculation (I_1)	58.23 C	17.66	111.45 B	1.89	0.78	14.58 B	26.97 C
Soil inoculation (I_2)	61.86 B	18.88	115.59 AB	2.02	0.85	15.73 A	28.68 B
Seed inoculation (I_3)	65.87 A	20.33	120.08 A	2.13	0.93	16.50 A	29.87 A
HSD (≤ 0.05)	2.98	1.58	7.35	0.04	0.02	0.83	0.80
Interaction (N × I)	NS	Fig. 3*	NS	Fig. 4*	Fig. 5*	NS	NS
HSD (≤ 0.05)		3.79		0.10	0.05		

Means sharing different letters within a column differ from each other at p≤0.05

NS = non-significant; * = Significant at p≤0.05

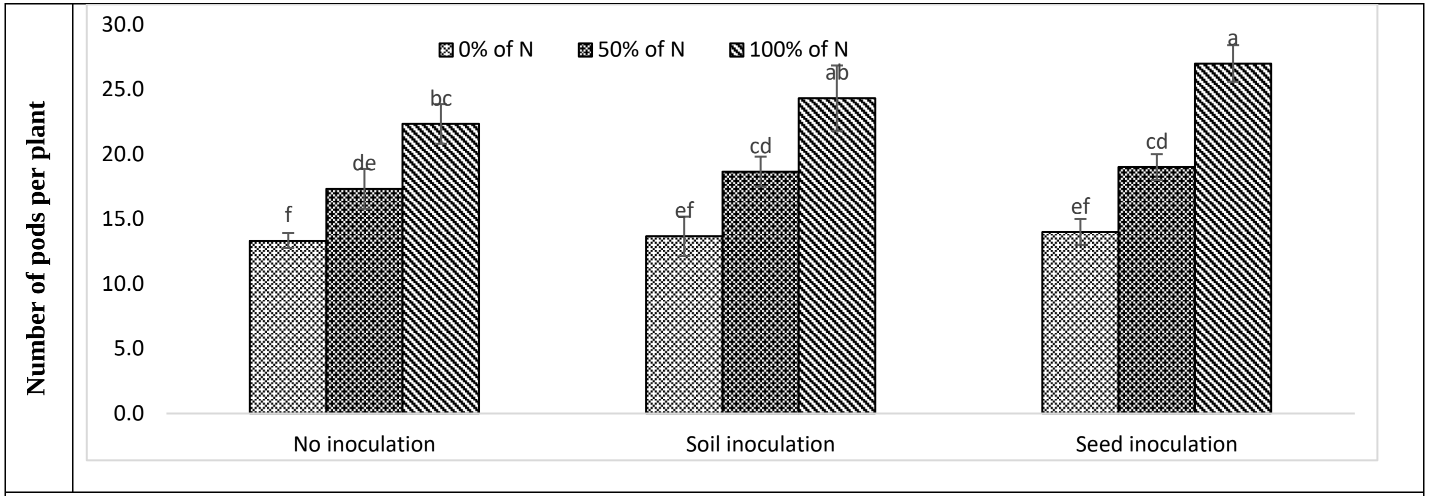

Fig. 3: Interactive effect of nitrogen rates and inoculation techniques on number of pods per plant of soybean.

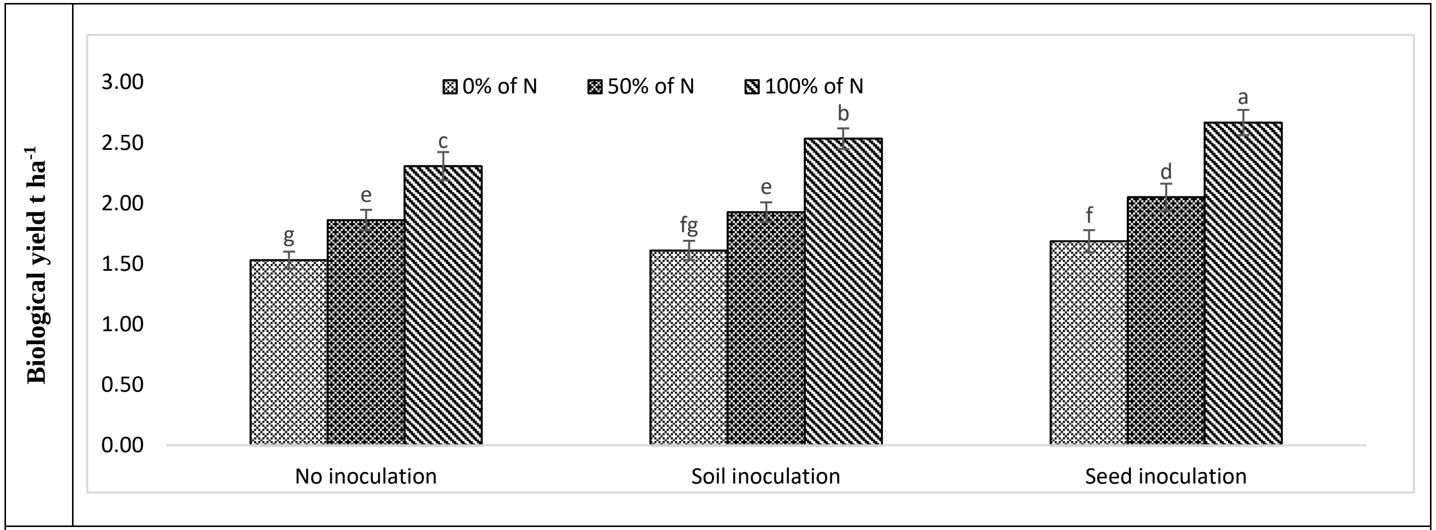

Fig. 4: Interactive effect of nitrogen rates and inoculation techniques on biological yield t ha^{-1} of soybean.

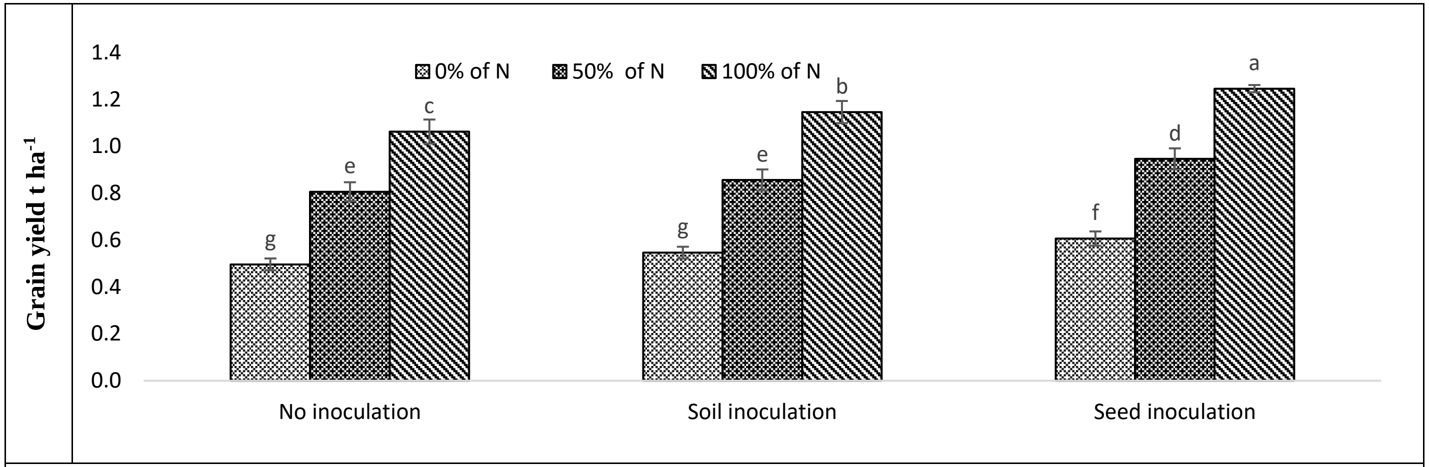

Fig. 5: Interactive effect of nitrogen rates and inoculation techniques on grain yield t ha^{-1} of soybean.

Table 3: Economic analysis of maize-soybean intercropping system

Treatments	**a. Maize grain income (Rs.)**	**b. Maize Straw income (Rs.)**	**c. Maize Gross Income (Rs.) c = a + b**	**d. Soybean income (Rs.)**	**e. Gross income (Rs.) (Maize + Soybean) e = c + d**	**f. Total expenditures in hectare (Rs.) Maize + Soybean**	**g. Net income (Rs.) e –f**	**BCR e/f**
N_1I_1	68437.5	9810	78247.5	25000	103247.5	165028.75	- 61781.25	0.62
N_1I_2	70625	10160	80785	27500	108285	165028.75	-56743.75	0.65
N_1I_3	73437.5	10350	83787.5	30500	114287.5	165028.75	-50741.25	0.69
N_2I_1	127187.5	11870	139057.5	40500	179557.5	172895.70	6661.8	1.03
N_2I_2	134687.5	11810	146497.5	43000	189497.5	172895.70	16601.8	1.09
N_2I_3	140937.5	11820	152757.5	47500	200257.5	172895.70	27361.8	1.15
N_3I_1	154375	11910	166285	53000	219285	180762.65	38522.35	1.21
N_3I_2	169062.5	11780	180842.5	57500	238342.5	180762.65	57579.85	1.31
N_3I_3	179687.5	11660	191347.5	62500	253847.5	180762.65	73084.85	1.40