



Sustainable maize production through seed inoculation and different tillage regimes

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Abstract

Maize is ranked as the third important cereal crop used as staple food after wheat and rice across the globe. However, population is increasing very rapidly and land holdings sizes are decreasing. Moreover, increasing input prices are also forcing the farmers switch towards conservative production systems. This situation needs yield increase per unit area to meet the food demands of increasing population. In this scenario, use of bio fertilizers with proper soil tillage practices can improve agricultural productivity on sustainable basis. The present study was conducted to evaluate the impacts of various tillage practices (Conventional, deep tillage and zero tillage) and seed inoculation strains on growth, yield and quality of spring sown maize. The experiment was conducted in RCBD in split plot arrangement with three replications. Maize hybrid (DKC-6142) was used as test cultivar. Both the qualitative and quantitative parameters of maize were significantly affected by tillage regimes and inoculation strains. However, their interaction was found non-significant for all the studied traits. Yield related traits and grain yield was significantly better recorded when maize seeds were inoculated and non-inoculated maize seeds performs poor in term of all these parameters. However, maize seeds sown without application of inoculum produced highest oil starch contents. Among the tillage practices, yield related traits, grain yield and protein contents were significantly better in conventional tillage followed by deep tillage, while performance of maize sown under zero tillage was poor in terms of these traits. However, crop sown under zero tillage produced highest oil and starch contents. In crux, seed inoculation of maize seeds may be used as an option to increase the maize yields in conventional system to meet the food demands of ever increasing population.

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Introduction

Maize is known as the third most important cereal crop used as staple food after wheat and rice in many countries of the world. It is gaining more importance in our local cropping system because of its high yield, short growing season, forage for animals, feed for poultry and is raw material for many agro based industries (Saif *et al.*, 2003). Among the most yield contributing factors, tillage and nitrogen are considered to be main factors. There is almost 20% contribution of tillage in crop production (Ahmad *et al.*, 1996). Tillage improves soil fertility because of its role in soil biological processes. These days reduced tillage options are more preferred over conventional tillage options because of controlling erosion and resource conservation. Farm energy requirements and cost of production can be minimized by adoption of reduce tillage operations (Monzon *et al.*, 2006). In order to preserve soil structure and moisture contents more focus should be on conservation tillage (Samarajeewa *et al.*, 2006). Barzegar *et al.* (2003) reported that crop yield obtained by using reduced tillage was equivalent or higher than conventional tillage under different environmental conditions. Zero tillage system conserves soil organic matter because of its less oxidation in soil (Wilkins *et al.*, 2002).

Sustainable farming is being preferred over conventional farming because it is eco-friendly (Poudel *et al.*, 2002). To reduce environmental risks and increase crop productivity, there is need to rely on renewable resources and inputs which is basic principle of sustainable agriculture (Kizilkaya, 2008). Nitrogen is one of the macronutrient which is vital for plant growth and development and it has role in protein synthesis and nucleic acid formation. Bio fertilizers play an important function in sustainable agriculture through integrated nutrient management and also increase crop yield (Marchner, 1995). They fix atmospheric nitrogen and influence the growth of plant by secreting plant growth regulators (Zahir *et al.*, 2004). The effect of phyto-hormones is direct, as they stimulate root growth, providing more sites for infection and nodulation (Garcia *et al.*, 2004).

Inoculation with nitrogen fixing bacteria increased yield of cereals significantly (Ozturket *et al.*, 2003). Behl *et al.* (2003) used *Azotobacter* as inoculants and reported that increase in grain yield, 1000 grain weight and biological yield of wheat was recorded. Significant increase in growth and yield of agronomically important crops in response to inoculation with PGPR have been reported (Asghar *et al.*, 2002). There was 19.8% increase in yield in maize by inoculation with *Azotobacter* (Zahir *et al.*, 1998). Núñez *et al.* (2012) propose that tillage does not increase the certain bacterial groups but increases efficiency of some bacteria, illustrating that tillage has influence on the performance of the naturally occurring or inoculated bacteria. Valencia *et al.* (2004) also explained the effect of tillage practices on working of soil bacteria and claim that knowledge is lacking in perspectives of bacterial efficiency under various tillage practices. Research on various trends and uses of beneficial bacteria has been carried out but to best of our knowledge no such comparative study has been done on maize with different strains and tillage practices. This study was motivated to estimate and evaluate the effect of tillage practices on the performance of rhizobacteria. The present study was therefore, been planned to evaluate the qualitative and quantitative response of spring planted maize under different tillage practices and seed inoculation with nitrifying bacteria.

Material and Methods

Experimental site

The experiment was conducted on a sandy clay loam soil at the Agronomic Research Area, Department of Agronomy, University of Agriculture, Faisalabad (Pakistan). The climate of the region is semi-arid and subtropical.

Production technology

Experiment was conducted in Randomized Complete Block Design (RCBD) with split plot arrangement having three replications. Maize hybrid DKC-6142 was used as test cultivar. Seeds of maize were sown after inoculating with *Azotobacter* and *Azospirillum* strains in the field prepared by various tillage practices. In deep tillage chisel plough was operated

in the field followed by two cultivations and two plankings. In conventional tillage, 3 cultivations were carried out followed by two plankings. In zero tillage, maize weeds were directly drilled into the soil without soil preparation. The net plot size was 3 m x 6 m. Crop was sown on 28th February, 2012 by single row hand drill by maintaining row to row distance of 60 cm and plant to plant distance of 20 cm using seed rate of 25 kg ha⁻¹. Fertilizer was applied at recommended rate of 125-120-150 kg ha⁻¹ of N-P-K respectively. All phosphorus and potash was applied at the time of sowing while nitrogen was applied in three splits, 1st at sowing, 2nd at tasseling, and 3rd at grain formation stage. About 8-10 irrigations were given when needed up to maturity. Other agronomic practices were kept uniform for all the treatments. Necessary plant protection measures were adopted to keep crop free of weeds, insect, pests and diseases.

Data Collection

The experiment comprised of three tillage practices (Zero Tillage, conventional tillage and deep tillage) and three seed inoculation treatments viz. Control, Inoculation with *Azospirillum* and Inoculation with *Azotobacter*. Standard procedures were followed to collect the data for growth and yield parameters. Ten plants from each plot were selected at random and their height was measured with the help of measuring tape and average was calculated. From each plot, ten cobs were selected and number of rows per cob and number of grains per cob were counted and averaged. At maturity crop was harvested and sun dried; overall biomass of each plot was obtained and converted to tone's per hectare. The cobs were shelled through maize sheller and grain yield per plot was calculated which was then converted to tone's per hectare. From each plot five samples of 1000-grains were collected randomly to record their weight and then averaged.

Harvest Index

Harvest index (HI) of each plot was calculated by using the formula:

$$HI = (\text{Economic yield} / \text{Biological yield}) \times 100$$

Grain quality analysis

Nitrogen content of maize seed sample collected from each subplot was determined by using the micro-Kjeldhal's method (Anonymous, 1990) and then the protein content was calculated by using the following formula i.e.

$$\text{Crude protein} = \text{Nitrogen} \times 6.25$$

Gluco-amylase method (Anonymous, 1990) was used to find out the starch contents from oven dried grain samples after grinding with grinder, while, Soxhelt method (Low, 1990) was used to determine the maize grain oil content.

Statistical analysis

The collected data was analyzed statistically by employing the Fisher's analysis of variance technique (Steel *et al.*, 1997) and treatment's mean was compared by using Least Significance Difference (LSD) test at 5% probability level.

Results

Plant height (cm)

Plant height was significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Regarding tillage practices, maximum plant height was (188.64 cm) recorded for T₁ which was statistically at par with T₂. Minimum plant height (178.84 cm) was recorded in T₀. For seed inoculation, maximum plant height was (190.13 cm) recorded for S₂ (*Azotobacter*) treatment which was statistically similar to S₁ (*Azospirillum*), while minimum plant height was (162.79 cm) recorded for control or untreated seed (Table 1).

Table 1: Effect of different tillage practices and PGPR inoculation on growth, yield and quality parameters of hybrid maize

	Deep tillage	Conventional tillage	Zero tillage	Means
Plant height (cm)				
Control	162.67	167.27	158.43	162.79 B
Azospirillum	184.47	194.53	170.53	183.18 A
<i>Azotobacter</i>	189.40	204.13	176.87	190.13 A
Means	178.84 AB	188.64 A	168.61 B	
LSD (p 0.05) Tillage = 15.49; Inoculation = 14.45				
Number of grain rows per cob				
Control	14.27	14.73	14.00	14.33 B
Azospirillum	16.07	17.07	15.13	16.09 A
<i>Azotobacter</i>	16.80	17.73	15.73	16.76 A
Means	15.71 AB	16.51 A	14.96 B	
LSD (p 0.05) Tillage = 1.33; Inoculation = 1.57				
Number of grains per cob				
Control	356.93	368.93	342.93	356.27 B
Azospirillum	403.80	452.73	378.73	411.76 A
<i>Azotobacter</i>	422.93	481.13	387.80	430.62 A
Means	394.56 AB	434.27 A	369.82 B	
LSD (p 0.05) Tillage = 63.11; Inoculation = 31.67				
1000 grain weight (g)				
Control	237.47	242.30	228.18	235.98 B
Azospirillum	263.88	281.87	248.63	264.79 AB
<i>Azotobacter</i>	272.52	298.95	255.10	275.52 A
Means	257.96 AB	274.37 A	243.97 B	
LSD (p 0.05) Tillage = 25.91; Inoculation = 29.00				
Biological yield (t ha⁻¹)				
Control	14.75	15.02	14.48	14.75 B
Azospirillum	15.82	16.25	15.31	15.79 A
<i>Azotobacter</i>	16.06	16.41	15.56	16.01 A
Means	15.54 AB	15.89 A	15.12 B	
LSD (p 0.05) Tillage = 0.56; Inoculation = 0.33				
Grain yield (t ha⁻¹)				
Control	6.26	6.50	6.10	6.29 B
Azospirillum	7.17	7.60	6.67	7.15 A
<i>Azotobacter</i>	7.38	7.75	6.91	7.34 A
Means	6.94 AB	7.28 A	6.56 B	
LSD (p 0.05) Tillage = 0.57; Inoculation = 0.33				

	Deep tillage	Conventional tillage	Zero tillage	Means
Harvest index (%)				
Control	42.44	43.25	42.10	42.59 B
Azospirillum	45.29	46.76	43.54	45.19 A
<i>Azotobacter</i>	45.90	47.18	44.37	45.82 A
Means	44.55	45.73	43.34	
LSD (p 0.05) Inoculation = 1.23				
Grain protein contents (%)				
Control	8.19	8.36	7.98	8.18B
Azospirillum	8.67	9.02	8.46	8.72 A
<i>Azotobacter</i>	8.82	9.19	8.54	8.85 A
Means	8.56 AB	8.86 A	8.3278 B	
LSD (p 0.05) Tillage = 0.32; Inoculation = 0.3635				
Grain oil contents (%)				
Control	5.57c	5.61bc	5.79a	5.66 A
Azospirillum	5.18ef	5.35d	5.70ab	5.41 B
<i>Azotobacter</i>	5.11f	5.27de	5.74a	5.37 B
Means	5.28 C	5.41 B	5.74 A	
LSD (p 0.05) Tillage = 0.05; Inoculation = 0.06 ; Tillage × Inoculation = 0.1054				
Grain starch contents (%)				
Control	63.90	64.56	69.80	66.09 A
Azospirillum	61.16	62.93	66.40	63.50 B
<i>Azotobacter</i>	60.23	61.86	67.30	63.13 B
Means	61.77 B	63.12 B	67.83 A	
LSD (p 0.05) Tillage = 2.21; Inoculation = 3.218				

Number of grain rows per cob

Number of grain rows per cob were significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Regarding tillage practices, maximum number of grain rows were (16.51) recorded in T₂, which was statistically at par with T₁. Minimum number of grains per cob was (14.96) recorded in T₀. Regarding seed inoculation maximum number of grain rows per cob were (16.76) in S₂ (*Azotobacter*) which was statistically similar to S₁ (*Azospirillum*) while minimum number of grain rows per cob were (14.33) recorded in S₀ (control) (Table 1).

Number of grains per cob

Number of grains per cob significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Among tillage practices, T₁ produced maximum number of grains per cob (434.27) which was statistically at par to T₂. Mean minimum number of grains per cob were (369.82) observed in T₀. Regarding seed inoculation, mean maximum number of grains per cob were (430.62) recorded in S₂ (*Azotobacter*) which was statistically similar to S₁ (*Azospirillum*). Mean minimum number of grain per cob were (356.27) recorded in S₀ (control). The interactive effect was found to be non-significant (Table 1).

1000-grain weight (g)

1000 grain weight significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Regarding tillage, maximum 1000 grain weight was (274.37g) recorded in T₂ which was statistically at par to T₁, while mean minimum 1000 grain weight was (243.97g) recorded for T₀ treatment. For seed inoculation, maximum 1000 grain weight was (275.52g) recorded for seeds inoculated with *Azotobacter* which was statistically similar to the seeds inoculated with *Azospirillum* while minimum 1000 grain weight was (235.98g) recorded for seeds sown without inoculation. The interaction of both factors showed non-significant effect on 1000 grain weight (Table 1).

Biological yield (t ha⁻¹)

Biological yield was significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant (Table 1). Data elaborated that for tillage factor, maximum biological yield was (15.89 t/ha) observed for T₁ which was statistically at par to T₂ while minimum biological yield was (15.12 t/ha) observed in T₀. Regarding seed inoculation, maximum biological yield was (16.01 t/ha) recorded for seeds initially inoculated with *Azotobacter* which was statistically similar to *Azospirillum* treated seeds while mean minimum biological yield was (14.75 t/ha) recorded for seeds sown without inoculums. The interaction of both factors was non-significant (Table 1).

Grain yield (t ha⁻¹)

Grain yield was significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Regarding tillage, mean maximum grain yield was (7.28 t/ha) recorded in T₂ which was statistically at par to T₁ while minimum grain yield was (6.94 t/ha) recorded in T₀. For seed inoculation, maximum grain yield was (7.34 t/ha) recorded where the seeds were treated with *Azotobacter* which was statistically similar where seeds were inoculated with *Azospirillum* while mean minimum grain yield was (6.29 t/ha) recorded in

non-inoculated seeds. The interactive effect of different tillage practices and seed inoculation was non-significant for grain yield of maize crop (Table 1).

Harvest Index

Harvest index was not significantly affected by tillage practices but seed inoculation affected the harvest index significantly. The interaction of tillage methods with seed inoculation was found non-significant. For seed inoculation mean maximum harvest index was (45.82%) observed in S₂ which is statistically similar to S₁ while minimum harvest index was (42.59%) recorded in S₀ (control). The interactive effect was found non-significant (Table 1).

Protein contents in grain (%)

Grain protein contents in maize were significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Regarding tillage, mean maximum protein contents were (8.86%) observed in T₂ which was statistically at par to T₁ while mean minimum protein contents were observed in T₀. For treatments where seeds were inoculated with bacteria, maximum protein contents were (8.85%) observed in S₂ which is statistically similar to S₁, while mean minimum protein contents were (8.18%) recorded in S₀ (control). The interactive affect was non-significant (Table 1).

Oil contents in grain (%)

Oil contents were significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Regarding tillage, mean maximum oil contents were (5.74%) recorded in T₀ followed by (5.41%) in T₂ while mean minimum oil contents were (5.28%) in T₁. For seed inoculation, maximum oil contents were (5.66%) recorded in S₀ followed by (5.41%) in S₁ while minimum oil contents were (5.37%) recorded in S₂. The interaction of tillage and seed inoculation was also significant on grain oil contents. Significantly maximum oil contents were recorded in plots where zero tillage and no seed inoculation were done (Table 1).

Starch contents in grain (%)

Starch contents were significantly affected by tillage practices and seed inoculation. However, the interaction of tillage methods with seed inoculation was found non-significant. Regarding tillage, mean maximum starch contents were (67.83%) recorded in T₀ followed by (63.12%) in T₂ while mean minimum starch contents were (61.77%) recorded in T₁. For starch contents in grain, non-significant difference was observed between seed inoculation treatments. The interaction effect of tillage and seed inoculation on starch contents was non-significant (Table 1).

Discussion

Various tillage practices and seed inoculation with both strains significantly affected the grain yield and quality of maize. Growth of maize was poor in non-inoculated seeds as compared to PGPR treated seeds which might be ascribed to less availability of nitrogen to maize in control treatment. However, both strains improved grain yield and were statistically at par with each other. Soil loosening plays a considerable role in improving crop performance; however, extent of crop performance depends on tillage type. In our study three tillage methods were employed to check the yield improvement of inoculated and non-inoculated maize. Results depicted that improvement in contribution of each yield contributing parameter towards fine grain yield varied among tillage systems. All quantitative parameters performed higher under conventional tillage system followed by deep tillage and were poor in zero tillage (Table 1). Several studies are in support of our findings and reported yield maximization in case of soils where tillage is applied because of the availability of nutrients and deep root system as compared to soils in which tillage was not applied (Albuquerque *et al.* 2001; Rashidi and Keshavazpour, 2007). Higher number of grain rows per cob in tilled soils might be due to presence of favorable conditions for growth, developed root systems and more nutrients uptake. In zero tillage minimum plant height is due to late emergence of seedlings and short roots because soil compactness was more and resulted in lower grain yield of maize (Pommel *et al.*, 2002). Higher number of grain rows

per cob has already been reported in soils where tillage was applied by Rashidi and Keshavazpour (2007). The results of 1000 grain weight are in line with Albuquerque *et al.* (2001) who reported that plant height, number of grains per cob and grain weight were higher in conventional tillage as compared to zero tillage system. Malakuti and Tehrani (2001) reported that seed inoculation with *Azotobacter* and *Azospirillum* produces heavier 1000 grain weight than untreated seeds. The results of biological yield are in line with Karunatilake (2000) who reported that in zero tillage biological yield is lowered due to higher soil compactness and unfavorable conditions for root growth and lower nutrient uptake. Similarly, Marwat *et al.* (2007) reported that conventional tillage systems are more productive than zero and reduced tillage systems

Inoculation with PGPR is reported as a sustainable approach, improves yield via synthesizing phytohormones which increase the availability of nutrients and also enhance their availability to plants through prolonged root system (Burdet *et al.* 2000). In our study, PGPR treated maize performed well than control plot. Parameters under study like plant height, number of grain rows per cob, number of grains per cob and 1000 grain weight were observed higher in inoculated maize, as reported by various studies. Seed inoculation also increases the availability of nutrients through extensive root systems which are utilized efficiently by plant. In a study, Burd *et al.* (2000) reported that seed inoculation with PGPR increased yield and yield attributing components. Our results of number of grains per cob are also in line to Albuquerque *et al.* (2001) who reported that plant height, number of grains per cob were reduced in case of zero tillage as compared to conventional tillage. These results also coincide with Gholami *et al.* (2009) who reported that increased number of grains per cob through inoculation with PGPR might be due to positive response of corn to seed inoculation. Moreover, Lucangeli and Bottini (1997) reported that seed inoculation with *Azospirillum* and *Azotobacter* resulted in production of certain plant growth regulators such as auxin which results in cell division

and also production of other growth promoting substances which ultimately increased grain yield. In another study, Naserirad *et al.* (2011) reported that bio-fertilizers use can increase harvest index due to its largely effect on dry matter and thus more assimilates are translocated to grain.

Quality parameters were highly variable among inoculation treatments as there was significant effect of inoculation on protein content, whereas oil contents were high in control plot. Non-significant effect of inoculation on improving starch content might be ascribed to more utilization of N in maize growth as confirmed by Zhang *et al.* (2010) who reported that absorption of more nitrogen by maize plants resulted in lower starch contents. In case of tillage regimes, more starch contents and oil contents were scored by T₀ (Zero tillage), might be ascribed to lower nitrogen availability in zero tillage. These results are in accordance with Cociu and Alionte (2011) who studied that maize crop sown in zero tillage had maximum oil contents as compared to on tilled soil. This finding can be attributed to the fact that tillage enhances availability of nutrients due to prolonged root system. These results are however, contradictory to the study conducted by Stefan *et al.* (2013) who reported that seed inoculation with PGPR increases carbohydrates content of grain in runner bean. While, protein contents were more in T₁ (conventional tillage), increase in protein content might be attributed to availability and more uptake of nitrogen due to more root proliferation in soil. These results are confirmed by Vita *et al.*, (2007) who reported that grain protein contents were higher under conventional tillage as compared to zero tillage. These protein contents were affected by prolonged root systems and biomass which increase nutrient uptake especially nitrogen which is building block of amino acid which contribute to protein formation. These results are also supported by the results obtained by Bashan *et al.*, (2004) who reported that protein contents were higher in inoculated plots due to more nitrogen availability.

Conclusion

Seed inoculation with PGPR accomplished with conventional tillage provides a pragmatic option to improve hybrid maize productivity in conventional systems.

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