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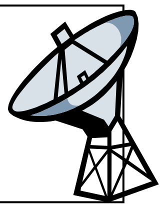

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Contents

Evaluation of Different Tillage and Crop Establishment Methods for Wheat Cultivation in Rice-Wheat System in the Terai Region of Nepal

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ABSTRACT

Tillage and crop establishment methods, residue management and nitrogen levels affect the crop and water productivity and profitability of wheat production. Field experiments to evaluate conventional tillage (CT), permanent raised bed (PRB) and zero tillage (ZT) under residue retention and residue removal with three nitrogen levels (0, 100 and 120 kg N ha^{-1}) in wheat on productivity, irrigation water saving and productivity, and profitability were carried out from 2010 to 2012 in rice-wheat cropping system. The experiments were carried out in strip split plot design with three replications. Zero till wheat produced significantly higher grain yield (2616.5 kg ha^{-1}), irrigation water saving (34%), irrigation water productivity (3.35 kg m^{-3}), and B:C ratio (1.82), as compared to conventional tillage, over two years. Tillage and crop establishment costs were the lowest for ZT (NRs 2275.92 ha^{-1}) followed by PRB (NRs 5380.24 ha^{-1}) and CT (NRs 7012.5 ha^{-1}). While, PRB produced slightly lower grain yield (2163 kg ha^{-1}), but, the highest water saving (40.6 %), comparable water productivity (2.1 kg m^{-3}) and higher B:C ratio (1.55) than CT. Net income, NRs ha^{-1}, from ZT, PRB, and CT were 35733, 23641, and 15830, respectively.

Key words: Nitrogen levels, productivity, profitability, residues, tillage and crop establishment, water productivity

INTRODUCTION

The rice-wheat (RW) system is the lifeline of millions of food producers and consumers in the Indo-Gangetic Plains (IGP) of South Asia. Practiced over an area of 13.5 million ha, this irrigated system provides food, employment, and income to the local population of the four IGP countries-Bangladesh, India, Nepal, and Pakistan (Hobbs and Gupta 2003). The system supports more than 450 million people and contributes more than 80% of the total cereal production in these countries (Ladha et al 2003). But, the system yield is stagnating or even declining in South Asia (Ladha et al 2002). Total factor productivity is declining, and farmers have to apply more fertilizer to obtain the same yields (Hobbs and Morris 1996). Soil organic matter is also declining. From a few hectares in 1998 to more than 100,000 ha in 2001 (Hobbs and Gupta 2003), today, approximately 4 million ha of RW area is under resource conservation technologies (RCTs) in the IGP countries. The RCTs offer considerable potential for improving the sustainability and productivity of wheat in the rice-wheat system of South Asia. These include raising input use efficiency, cutting costs, providing various environmental benefits, and ultimately improving farmer livelihoods, and helping to reduce poverty (Hobbs and Gupta 2003).

Adoption of intensive cropping system, removal of residue for fodder or burning and frequent tillage operation to facilitate the emergence using mainly small, tractor-drawn disc plows/harrows and field cultivators are the common practices in the IGP area that leads to losses of soil, water, and nutrients and deteriorate soil by decreasing organic matter content and fragile physical structure, consequently reduce crop yields, water and fertilizer use efficiency. Using nitrogenous fertilizer efficiently reduces environmental problems and cost of production (Ladha et al 2003). Burning of residue releases greenhouse gases and particulate matter in large quantities in sudden spurts deteriorating air quality, resulting in significant losses of nutrients from the soil (Sharma and Mishra 2001) and has adverse effects on human health (Gupta et al 2003, Hobbs and Gupta 2003). The incorporation of wheat residues increased the yield of rice and also had a positive residual effect on the yield of subsequent wheat (Samra et al 2003).

Tillage is the second costliest monetary input after fertilizer for wheat production (Singh et al 2011). The use of raised beds for production of irrigated wheat in the rice-wheat areas of the IGP was pioneered during the 1990s, inspired by the success of beds for wheat-maize systems in Mexico (Sayre and Hobbs 2004). Rice and wheat are the fertility exhaustive and need more water, labor, time, non-renewable energy, heavy farm machineries and costs for their successful cultivation (Jha et al 2011) and many times frequent breakdown in farm machineries also poses serious problem in land preparation for timely sowing of succeeding crops (Gopal et al 2010). Traditionally, the establishment of the wheat crop includes repeated plowing (6-8), cultivating, planking, and pulverizing the topsoil. Repeated tillage operations delay planting, escalate costs, and reduce farmers' profit margins (Hobbs et al 1992; Harrington et al 1993a,b).

Wheat sowing can be accomplished efficiently with the use of improved machineries viz. zero till seed drill, rotary seed drill, and bed planter etc to save the time, diesel (60 liter ha^{-1} with zero tillage), energy, and cost (Jha et al 2007). Adopting zero tillage on even one million ha of rice-wheat area would reduce more than 156,000 Mg yr^{-1} CO_2 emissions, using a conversion factor of 2.6 kg CO_2 produced per liter of diesel burned (Hobbs and Gupta 2003). It is therefore, imperative to identify the suitable tillage and crop establishment methods of wheat cultivation in association with residue management in rice-wheat system for higher crop productivity, water use efficiency, and profitability in the terai region of Nepal.

MATERIALS AND METHODS

The field experiments were carried out at Pheta VDC in Bara district, Nepal for two years, from 2010/11 to 2011/12. The soil of the experimental field was slightly acidic (pH 5.7) silt loam, high in organic matter (4.98%), high in total N (0.241%), very high in P (379 kg ha^{-1}), and medium in exchangeable K (118 kg ha^{-1}) contents. The experiments consisted of three factors: (a) tillage and crop establishment (TCE) methods, (b) residue management, and (c) nitrogen levels for both crops under rice-wheat system and were conducted in strip-split plot design with three replications. The size of each plot was 37.8 m^2 (7 x 5.4 m) as seeding width of the ZT-drill was 1.8 m and of the furrow-irrigated raised bed (FIRB)-drill was 1.35 m. The TCE methods comprised of (i) conventional tillage (CT): plots were ploughed twice (double passes each time) using tractor-drawn cultivator followed by wooden planking, seed and basal fertilizers were manually broadcast on the tilled soil surface followed by shallow seed and soil manipulation with the cultivator followed by light planking; (ii) permanent raised bed (PRB): seeds were drilled, 5 cm deep, over rice harvested bed tops, in two rows, after superficial reshaping using furrow irrigated raised bed (FIRB) drill; and (iii) zero tillage (ZT): seeds were drilled, 5 cm deep, on untilled rice harvested plots using inclined plate zero-till seed drill.

The residue management consisted of (i) residue retention (R_R): 40 cm stubbles of preceding crop were left at harvest and (ii) residue removal (R_O): preceding crop was harvested from ground level leaving 5 cm stubbles. The nitrogen levels were: (i) zero nitrogen (N_O): 0 kg ha^{-1}; (ii) farmers' nitrogen (N_{100}): 100 kg N ha^{-1}; and (iii) abundant nitrogen (N_{120}): 120 kg N ha^{-1}. Of the nitrogen levels, half N was applied as a basal dose at sowing time and the remaining N in two equal split dozes applied after irrigation applications. Phosphorus (P_2O_5) and potassium (K_2O) were applied @ 60 kg ha^{-1} and 40 kg ha^{-1}, respectively, as basal doses at sowing. The sources of fertilizers were urea, triple super phosphate, and muriate of potash. Wheat variety 'Gautam' with seed @ 120 kg $ha^{-1}$for CT and ZT and 80 kg ha^{-1} for PRB, was sown on 10 December, 2010 and 11 December 2011. Pre-sowing irrigation was applied to ensure optimum soil moisture a week before sowing to all the plots. Two irrigations were applied on 22 and 53 days after sowing (DAS) during the crop cycles. Irrigation water was lifted from a shallow tube-well and was conveyed to the individual plots through a 10-cm diameter poly-vinyl chloride (PVC) pipe, using a diesel pump-set. The rate of flow (Q) was determined by the following equation (Michael and Ojha 1999):

Rate of flow (lit sec^{-1}) = (Volume of container, lit)/ (Time required to fill, sec)

The depth of irrigation water applied was 5 cm for CT and ZT and 5 cm below from bed-top in the furrows for PRB. The amount of irrigation water applied was calculated as water volume (m^3 ha^{-1}) using the following equation:

Irrigation water (m^3 ha^{-1}) = [Rate of flow (l sec^{-1}) x time (per plot, sec) x 10000 m^{-2}]/ Plot area (m^2) x 1000

Irrigation water (mm) = Irrigation water (m^3 ha^{-1})/10

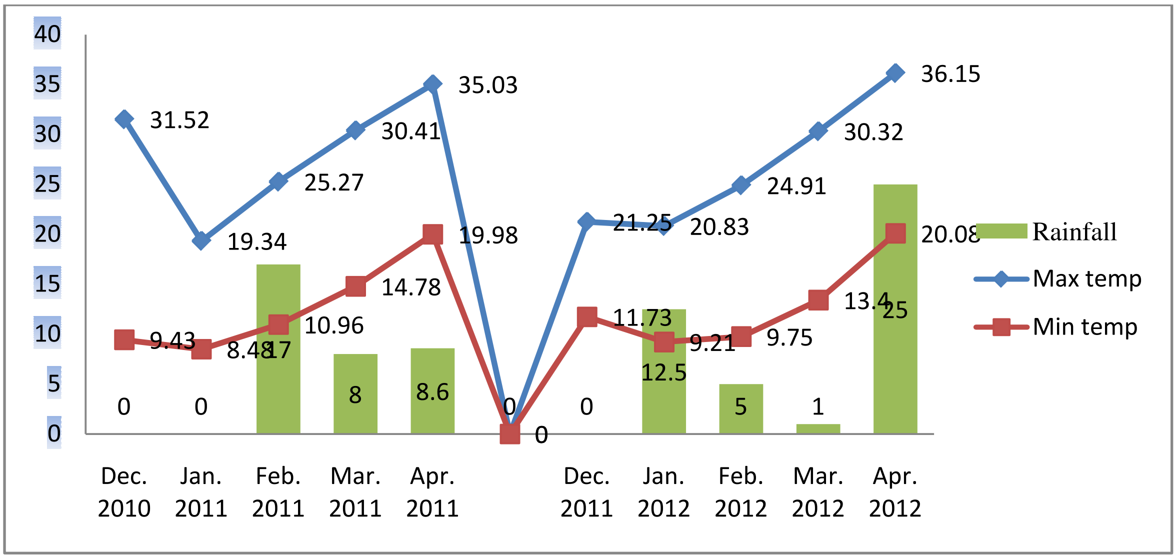
The total amount of applied water (input water) was calculated by summing irrigation water (m^3 ha^{-1}) and rainfall water (m^3 ha^{-1}). Rainfall data were recorded from a rain-gauge installed at the experimental site. The irrigation water productivity (WP_I) and input water productivity (WP_{I+R}) were calculated using the following equations (Alam et al 2014 unpublished, Laik et al 2014), where, considered effective rainfall was 5 mm or above at each spell:

WP_I (kg grain m^{-3} of irrigation water) = Grain yield (kg ha^{-1})/ Irrigation water volume (m^3 ha^{-1})

WP_{I+R} (kg grain m^{-3} of irrigation water + effective rainfall) = Grain yield (kg ha^{-1})/[(Irrigation water + effective rainfall water)(m^3 ha^{-1})]

For weed control, a mixture of Isoproturon + 2,4-D @ 900 g ha^{-1} each in 700 liter water was sprayed by using a knap-sack sprayer, at 35 DAS. The total rainfall received at the site during 1st and 2nd year were recorded 33.6 mm and 43.5 mm (Figure 1) in 3 and 6 spells (Figure 2), with effective rainfall of 33.6 and 38.5 mm, respectively. Human labor used for all operations and management practice, amounts of all inputs and outputs, pump set used for irrigation, input-output prices, wage rate and machinery rent were recorded for each plot. Grain and straw yields were determined manually harvesting five random samples (2 m^2 each for CT and ZT and 2.7 m^2 for PRB) from each plot at physiological maturity. Grain yields were adjusted at 12% moisture content. Straw yields were calculated on sun-dry weight basis. Benefit:cost (B:C) ratio analysis was done considering total variable cost (TVC) of crop production and gross return (GR).

The variable costs included costs of using human labor, machinery (tractor, cultivator, seed drills, pump-set, and thresher) rent, and the costs of inputs of production (seed, fertilizers, and chemicals). The cost of labor used for seed sowing, fertilizer broadcasting, irrigation, herbicide application for weed management, and harvesting of the crop by multiplying person-day ha^{-1} required for an operation with wage rate (Nepalese rupees, NRs person-day^{-1}). The number of labor and time (h) required to complete a particular field operation in each plot were recorded and was expressed as person-day ha^{-1}, considering 6 h to be equivalent to 1 person-day. Irrigation cost was calculated by multiplying rental rate (NRs h^{-1}) and pump-set used (h ha^{-1}). The threshing cost with the thresher was calculated as 10% of grain yield by multiplying farm gate price of wheat at harvest. The costs of inputs eg, seed, fertilizers, and chemicals were calculated based on prevailing market prices of the inputs. Gross returns (GR) were calculated by multiplying economic yields (grain and straw) of the crop with farm gate price at harvest time. Net returns (NR) were calculated as the difference between GR and TVC (NR= GR- TVC). The B:C ratio was calculated by dividing gross return with total variable cost. The mean values were computed and analyzed by Genestat 5.



Source : *Regional Agricultural Research Station, Parwanipur, Bara, Nepal.*

Figure 1. Maximum and minimum temperature (^{0}C) and rainfall (mm) during wheat growing at Pheta, Bara, Nepal, 2010/11-2011/12

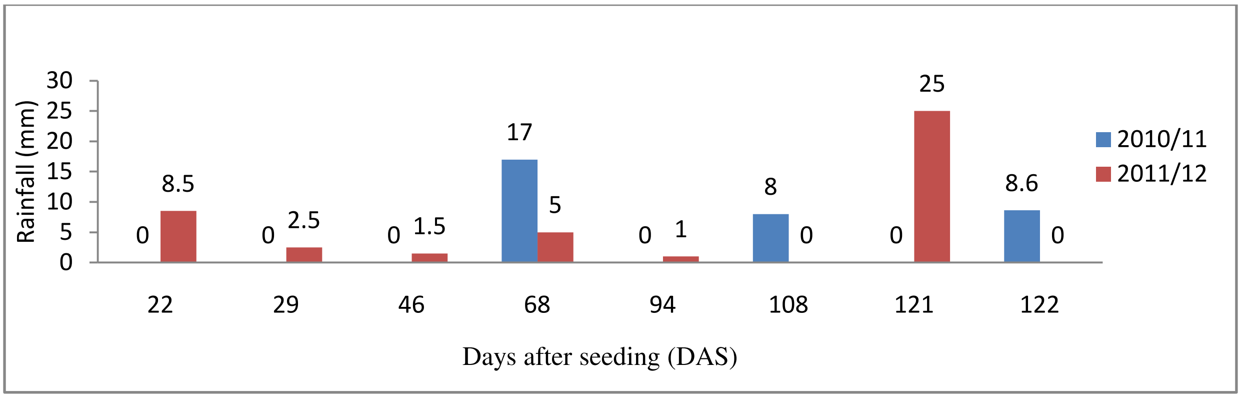


Figure 2. Rainfall distribution during wheat growing at Pheta, Bara, Nepal, 2010/11-2011/12

RESULTS AND DISCUSSION

Grain yields

Wheat grain yields were influenced significantly due to different tillage and crop establishment (TCE) methods during 2010/11 and 2011/12. The highest grain yields (2777 and 2456 kg ha^{-1}) were recorded from zero tillage compared to conventional tillage (2499 and 1964 kg ha^{-1}), and permanent raised bed (2438 and 1888 kg ha^{-1}) during the years, respectively. Hence, the mean grain yields, over the years, were significantly influenced by TCE methods. The highest mean grain yield (2616.5 kg ha^{-1}) was recorded from zero tillage (ZT) than CT (2231 kg ha^{-1}) and PRB (2163 kg ha^{-1}). Zero tillage produced higher grain yield than CT and PRB by 17.3% and 21.0%, respectively, while, CT and PRB were at par, statistically (Table 1). The higher grain yield with ZT, compared to CT, attributed to more tillers per unit area and higher specific weight of grain (Table 2), more increase in soil organic matter (0.11%) and enhanced decrease in soil bulk density (0.017 Mg m^{-3}) at second wheat harvest in 2011/12, and higher irrigation water productivity (Table 7). The results were in accordance with the results of Hobbs et al (1997) and Gupta et al (2000), that zero till wheat after rice produced yield that was equal to or even higher than those conventional crop. Melha et al (2000) also observed 6% higher yield in timely sown zero till wheat than the timely sown conventionally tilled wheat crop. The grain yield for TCE methods was lower in 2011/12, but, the percentage yield increase from ZT was higher. The lower grain yield on PRB, during the years, might be due to lower seed rate (80 kg ha^{-1}, 2/3rd of CT and ZT) resulting in lowered tillers per unit area (Table 2) and bed compaction with time.

The effect of residue retention (R_R) on grain yield, in 2010/11, was not encouraging, but, it improved in 2011/12. The effect of R_R on mean grain yield was positive and it increased the mean grain yield (2384.5 kg ha^{-1}) compared to residue removal $R_{0,}$ (2291.5 kg ha^{-1}). The increased yield from R_R in 2011/12 and the mean yield attributed to more number of grains per spike and specific weight of grain (Table 2), improved soil moisture and thermal regimes and improvement in the early vigor of wheat seedlings and subsequently system productivity (Tang et al 2000, Zheng 2000). The increased N dose significantly enhanced the wheat grain yields as well as the mean grain yields in both the years. Application of farmers' N dose (100 kg N ha^{-1}) and abundant N dose (120 kg N ha^{-1}) enhanced grain yield than control (0 kg N ha^{-1}), by (256 and 266%) and (252 and 275%) in 2010/11 and 2011/12, respectively. The higher grain yields from enhanced N dose attributed to more plant chlorophyll, plant vigor, more tillers, more leaf area index, more grains per spike and specific grain weight (Table 2). The results were in accordance with the findings of Ram (2000) and Balasubramanian et al (2000). The interaction effects between TCE methods and N doses on grain yields were significantly influenced in both the years (Table 2). Zero tillage without N application significantly produced the lowest grain yields (1128 and 914 kg ha^{-1}), while, the highest grain yields (3615 and 3294 kg ha^{-1}) were recorded from ZT with 120 kg ha^{-1}, in 1st and 2nd years, respectively. The interaction effects between TCE methods and residue and TCE method x N doses were significant for grain and straw yields, respectively, in 2011/12 (Table 3). Similarly, the interaction effects between TCE methods x residue x N doses were significantly influenced (Table 4). It is obvious that ZT without residue and N produced the lowest grain yield (765 kg ha^{-1}) and interestingly the highest (3329 kg ha^{-1}) without residue but with 120 kg N ha^{-1}.

Table 1. Wheat grain and straw yields as influenced by tillage and crop establishment methods, residues, and nitrogen levels at Pheta, Bara, Nepal, 2010/11-2011/12

Treatments	Grain yield (kg ha^{-1})			Straw yield(kg ha^{-1})		
	2010/11	2011/12	Mean over years	2010/11	2011/12	Mean over years
TCE methods:						
Conventional tillage	2499	1964	2231.5	2737	2720	2728.5
Permanent raised bed	2438	1888	2163.0	2299	2912	2606.0
Zero-tillage	2777	2456	2616.5	2846	3031	2939.0
LSD $_{(0.05)}$	216.2	98.0	125.3	429.5	273.3	305.5
F-test $_{(0.05)}$	**	**	**	*	NS	NS
Residue management						
Residue retention (R_R)	2499	2270	2384.5	2054	2758	2406.0
Residue removal (R_0)	2648	1935	2291.5	3201	3017	3109.0
LSD $_{(0.05)}$	176.5	80.0	102.3	350.7	223.1	249.5
F-test $_{(0.05)}$	NS	**	NS	**	*	**
Nitrogen level						
Control (N_0)	1239	1006	1122.5	1306	1525	1415.5
Farmer's N (N_{100})	3176	2532	2854.0	3284	3411	3347.5
Abundant N (N_{120})	3298	2770	3034.0	3292	3728	3510.0
LSD $_{(0.05)}$	216.2	98.0	125.3	429.5	273.3	305.5
F-test $_{(0.05)}$	**	**	**	**	**	**
CV (%)	12.4	6.9	7.9	24.1	14.0	16.4

**, significant at 5% level of significance. **, significant at 1% level of significance. NS, not significant. LSD, Least significant difference. CV, Coefficient of variance.*

Straw yield

Straw yields significantly varied in 2010/11, while, they were insignificant in 2011/12, from TCE methods. Zero tillage showed higher straw yields (2846 and 3031 kg ha^{-1}) compared to CT (2737 and 2720 kg ha^{-1}) and PRB (2299 and 2912 kg ha^{-1}), during the years, respectively. Zero tillage produced higher mean straw yield by 7.7% and 12.8% than CT and PRB, respectively. Residue removal showed significantly higher mean straw yield by 29.2% than R_R. For straw yields, R_R proved significantly superior in both the years. The increased N applications significantly enhanced straw yields in both the years (Table 1) because of increased plant height.

Table 2. Yield attributing characteristics of wheat as influenced by tillage and crop establishment methods, residue retention, and nitrogen levels at Pheta, Bara, Nepal, 2010/11-2011/12

Treatment	Number of effective tillers m^{-2}			Number of grains per spike			Thousand grain weight (g)		
	2010/11	2011/12	Mean	2010/11	2011/12	Mean	2010/11	2011/12	Mean
TCE methods									
Conventional tillage	163.8	167.7	165.75	41.32	31.45	36.38	44.31	36.68	40.50
Permanent raised bed	150.3	159.4	154.85	40.99	31.33	36.16	49.03	38.11	43.57
Zero-tillage	168.6	172.9	170.75	34.36	30.82	32.59	47.71	35.96	41.84
LSD $_{(0.05)}$	22.04	13.42	-	2.83	4.12	-	1.46	2.63	-
F-test $_{(0.05)}$	NS	NS	-	**	NS	-	**	NS	-
Residue management									
Residue retention (R_R)	152.9	174.7	163.8	39.04	32.24	35.64	46.47	39.17	42.82
Residue removal (R_0)	168.6	158.6	163.6	38.93	30.16	34.54	47.56	34.66	41.11
LSD $_{(0.05)}$	17.99	10.95	-	2.31	3.37	-	1.19	2.15	-
F-test $_{(0.05)}$	NS	**	-	NS	NS	-	NS	**	-
Nitrogen level									
Control (N_0)	136.8	153.5	145.15	31.32	25.58	28.45	47.94	33.61	40.78
Farmer's N (N_{100})	181.2	179.8	180.50	42.62	34.54	38.58	47.03	38.71	42.87
Abundant N (N_{120})	164.6	166.7	165.65	43.02	33.48	38.25	46.07	38.43	42.25
LSD $_{(0.05)}$	22.04	13.42	-	2.83	4.1	-	1.46	2.63	-
F-test $_{(0.05)}$	*	**	-	**	**	-	*	**	-
CV (%)	20.2	11.9	-	10.7	19.5	-	4.6	10.5	-

**, significant at 5% level of significance. **, significant at 1% level of significance. NS, not significant. LSD, Least significant difference. CV, Coefficient of variance.*

Table 3. Interaction effects of tillage and crop establishment methods x nitrogen levels and tillage and crop establishment methods x residues on wheat grain and straw yields at Pheta, Bara, Nepal, 2010/11- 2011/12

TCE methods	Grain yield (kg ha^{-1})								Straw yield (kg ha^{-1})		
	2010/11			2011/12			2011/12		2011/12		
	N_0	N_{100}	N_{120}	N_0	N_{100}	N_{120}	R_R	R_O	N_0	N_{100}	N_{120}
Conventional tillage	1318	2966	3212	1002	2295	2596	2195	1733	1509	3182	3469
Permanent raised bed	1271	2975	3067	1102	2140	2421	2127	1648	1747	3272	3718
Zero-tillage	1128	3587	3615	914	3160	3294	2489	2423	1319	3778	3996
LSD $_{(0.05)}$		374.5			169.7		138.6			473.3	
F-test $_{(0.05)}$		*			**		**			*	
CV (%)		12.4			6.9		6.9			14.0	

**, significant at 5 % level of significance. **, significant at 1 % level of significance. LSD, Least significant difference. CV, Coefficient of variance.*

Table 4. Interaction effects of tillage and crop establishment methods x residues x nitrogen levels on grain yields at Pheta, Bara, Nepal, 2011/12

TCE methods	Grain yield(kg ha^{-1})					
	Residue retention			Residue removal		
	N_0	N_{100}	N_{120}	N_0	N_{100}	N_{120}
Conventional tillage	1089	2698	2798	914	1893	2393
Permanent raised bed	1252	2454	2676	952	1827	2165
Zero-tillage	1064	3144	3259	765	3175	3329
LSD $_{(0.05)}$			240			
F-test $_{(0.05)}$			**			
CV (%)			6.9			

***, Significant at 1% level of significance. LSD, Least significant difference. CV, Coefficient of variance.*

Irrigation time

The irrigation application time was influenced by TCE methods during first irrigation in both the years and also during second irrigation in 2011/12 (Table 5). The total pumping time in PRB was the lowest (41 and 45.5 h ha^{-1}) compared to CT (70.7 and 72.8 h ha^{-1}) and ZT (46.6 and 52.1 h ha^{-1}) during 2010/11 and 2011/12, respectively. Therefore, the savings in pumping time over CT were (42% and 37.5%) from PRB and (34.1% and 28.4%) from ZT during 2010/11 and 2011/12, respectively. The mean saving in pumping time, over two years compared to CT, from PRB and ZT were 39.8% and 31.3%, respectively. The effects of R_R on applied irrigation time at both the irrigations in both the years were not significant. However, R_0 recorded lower irrigation application time compared to R_R. The reason for higher irrigation application time from residue retained field might be due to the lower velocity of recessing water at irrigation because of residues on soil surface.

Table 5. Irrigation time savings as influenced by tillage and crop establishment methods and residues in wheat season at Pheta, Bara, Nepal, 2010/11-2011/12

Treatments	Irrigation time (h ha^{-1})								
	1^{st} Irri.	2^{nd} Irri.	Total pumping time	Pumping time saving (%)	1^{st} Irri.	2^{nd} Irri.	Total pumping time	Pumping time saving (%)	Saving in pumping time over two years (%)
	2010/11				2011/12				
TCE methods									
Conventional tillage	50.0	20.7	70.7	-	43.7	29.1	72.8	-	-
Permanent raised bed	20.6	20.4	41.0	42.0	28.0	17.5	45.5	37.5	39.75
Zero-tillage	28.9	17.7	46.6	34.1	34.7	17.4	52.1	28.4	31.25
LSD $_{(0.05)}$	8.6	3.32	-	-	1.43	2.1	-	-	-
F-test $_{(0.05)}$	**	NS	-	-	**	**	-	-	-
Residue management									
Residue retention (R_R)	36.2	18.7	54.9	-	35.8	21.4	57.2	-	-
Residue removal (R_0)	30.0	20.5	50.5	8.0	35.1	21.3	56.4	1.4	4.7
LSD $_{(0.05)}$	7.02	2.7	-		1.2	1.68	-	-	
F-test $_{(0.05)}$	ns	ns	-		ns	ns	-	-	
CV (%)	38.3	25	-						

**, significant at 5% level of significance. **, significant at 1% level of significance. ns, not significant. LSD, Least significant difference. CV, Coefficient of variance.*

Irrigation water application

The applied irrigation water was significantly varied in all TCE methods of first irrigation 2010/11 and first and second irrigation of 2011/12 (Table 6). Total irrigation water application was the highest from CT (1272 and 1018 m^3 ha^{-1}) followed by ZT (839 and 715 m^3 ha^{-1}) and the lowest from PRB (738 and 618 m^3 ha^{-1}), during 1^{st} and 2^{nd} year, respectively, and followed the trend of CT>ZT>PRB. Compared to CT, ZT produced 17.3% higher mean grain yields and saving 31.9% irrigation water, whereas, PRB showed 3% lower grain yield saving 40.6% irrigation water. The results were in accordance with the results of Singh et al (2011). The effect of residue management on irrigation water application was not significant during the years.

Water productivity

Water is natural resource and its scarcity is seriously felt in agriculture sector. Irrigation water productivity was affected significantly due to various TCE methods, where ZT produced remarkable higher grain yield (3.3 and 3.4 kg) over CT (2.0 and 1.9 kg) and PRB (3.3 and 3.1 kg) with each m^3

water used, during 2010/11 and 2011/12, respectively (Table 7). Similar results were reported by Singh et al (2011). The higher irrigation water productivity from ZT could be due to higher grain yield and lower water used. Irrigation water productivity from PRB was equally good. Likewise, input water productivity from ZT was higher (2.4 and 2.23 kg) than PRB (2.3 and 1.88 kg) and CT (1.6 and 1.4 kg) per m^3 water used. The irrigation and input water productivities were enhanced by R_0 than R_R in 1st year, but, the result was reversed in 2nd year (Table 7).

Table 6. Irrigation water savings as influenced by tillage and crop establishment methods and residues in wheat season at Pheta, Bara, Nepal, 2010/11-2011/12

Treatments	Irrigation water (m^3 ha^{-1})								
	1st Irri.	2nd Irri.	Total water	Saving (%) over CT, R_R	1st Irri.	2nd Irri.	Total Water	Saving (%) over CT, R_R	Savings over years (%)
	2010/11				2011/12				
TCE methods									
Conventional tillage	899	373	1272	-	493	525	1018	-	-
Permanent raised bed	371	367	738	42.0	302	316	618	39.3	40.6
Zero-tillage	520	319	839	34.0	402	313	715	29.8	31.9
LSD $_{(0.05)}$	154.8	59.8	-	-	22.8	37.1	-	-	-
F-test $_{(0.05)}$	**	NS	-	-	**	**	-	-	-
Residue management									
Residue retention (R_R)	652	337	989	-	413	385.4	798.4	-	-
Residue removal (R_0)	541	369	910	8	385	383.5	768.5	3.7	5.8
LSD $_{(0.05)}$	127.4	48.8	-	-	18.6	30.3	-	-	-
F-test $_{(0.05)}$	NS	NS	-	-	*	NS	-	-	-

**, Significant at 5% level of significance. **, Significant at 1% level of significance. NS, not significant. LSD, Least significant difference.*

Table 7. Irrigation water productivity and input water productivity as affected by tillage and crop establishment methods and residues in wheat season at Pheta, Bara, Nepal, 2010/11- 2011/12

Treatments	Irrigation water		Rainfall water		Input water		Irrigation water productivity		Input water productivity	
	(m^3 ha^{-1})						(kg m^{-3})			
	2010/11	11/12	10/11	11/12	10/11	11/12	10/11	11/12	10/11	11/12
TCE methods										
Conventional tillage	1272	1018	336	385	1608	1403	2.0	1.9	1.6	1.40
Permanent bed planting	738	618	336	385	1074	1003	3.3	3.1	2.3	1.88
Zero-tillage	839	715	336	385	1175	1100	3.3	3.4	2.4	2.23
Residue management										
Residue retention	989	798	336	385	1325	1183	2.5	2.8	1.9	1.92
Residue removal	910	768	336	385	1246	1153	2.9	2.5	2.1	1.68

Note: considered effective rainfall = 5 mm and above at each spell.

Benefit:cost analysis

Benefit:cost (B:C) analysis of the treatments showed relevance to consider the practical adoptability of a particular treatment from the farmers' point of view. The data on economics (Table 8) revealed that ZT gave the highest gross return ha^{-1} (NRs 79,462) followed by CT (NRs 68,436) and the lowest by PRB (NRs 66,219). While, the highest production cost ha^{-1} was associated with CT (NRs 52,606) followed by ZT (NRs 43,729) and the least by PRB (NRs 42,578). Obviously, the highest net return ha^{-1} was recorded from ZT (NRs 35,734) followed by PRB (NRs 23,641) and the least from CT (NRs 15,830). Consequently, B:C ratio was the highest from ZT (1.8) followed by PRB (1.6) and the lowest from CT (1.3). The B:C ratio from R_0 compared to R_R was slightly higher. It was interesting to note that without N application, CT showed a monetary loss (NRs 5,282 ha^{-1}) with the lowest B:C ratio

(0.87). The highest B:C ratio (1.84) was obtained from 120 kg ha^{-1} N application and a comparable B:C ratio (1.78) was recorded from 100 kg ha^{-1} N applied. The results were in agreement with the findings of several researchers (Jha et al 2011, Laik et al 2014, Singh et al 2011).

Table 8. Profitability of tillage and crop establishment methods, residues, and nitrogen levels at Pheta, Bara, Nepal, 2010/11-2011/12

Treatments	NRs ha^{-1}			B:C ratio
	Gross returns	Production costs	Net return	
TCE methods				
Conventional tillage (CT)	68436.5	52605.9	15830.6	1.30
Permanent raised bed (PRB)	66219.0	42578.1	23641.0	1.55
Zero-tillage (ZT):	79462.5	43728.9	35733.7	1.82
Residue management				
Residue retention (R_R)	71599.5	47481.9	24117.7	1.51
Residue removal (R_0)	71197.5	45126.7	26070.8	1.58
Nitrogen level				
Control (N_0)	34554.0	39836.3	-5282.3	0.87
Farmer's N (N_{100})	87100.5	48859.1	38241.4	1.78
Abundant N (N_{120})	92448.0	50217.5	42230.5	1.84

Operation-wise variable cost

The lowest tillage and crop establishment cost ha^{-1} was obtained from ZT (NRs 2,275.9) followed by PRB (NRs 5,380.2) and the highest from CT (NRs 7,012.5). The irrigation cost was the highest from CT (24.8%) followed by ZT (19.9%) and the lowest from PRB (18.5%). Fertilization cost was the highest from PRB (24.8%) followed by ZT (23.7%) and the lowest from CT (20.0%). Other costs of operations are presented in Table 9.

Table 9. Wheat production costs (NRs ha^{-1}) as affected by farm operations under different tillage and crop establishment methods at Pheta, Bara, Nepal, 2010/11 - 2011/12

Particulars	Conventional tillage		Permanent raised bed		Zero tillage	
	Cost	(%)	Cost	(%)	Cost	(%)
Tillage and crop establishment	7012.5	12.9	5380.2	12.3	2275.9	5.0
Seeds and sowing	6840.0	12.6	4000.0	9.1	6000.0	13.1
Fertilizers	10877.0	20.0	10877.0	24.8	10877.0	23.7
Herbicide	2181.0	4.0	2181.0	5.0	2181.0	4.8
Irrigation	13475.0	24.8	8105.0	18.5	9135.0	19.9
Harvesting	6300.0	11.6	6300.0	14.4	6300.0	13.7
Threshing	7711.2	14.2	6962.0	15.2	9113.9	19.9
Total costs	54396.7	100	43805.2	100	45882.8	100

Note: Residue costs not included; Irrigation applied using a pump set; Farmers' N dose (100 kg Nha^{-1}) used here.

CONCLUSION

Results of this experiment indicated that zero-till (ZT) wheat cultivation could be successfully grown with an increased yield (17%), savings on irrigation time and water (32%), increased irrigation water productivity (3.35 kg m^{-3}), and higher B:C ratio (1.82), over conventional tillage (CT). Wheat cultivation on permanent raised bed (PRB) is also superior to CT for irrigation time and water savings

(40%) and higher B:C ratio (1.55). Forty cm residue retention (R_R) of preceding crops enhanced 4% grain yield than residue removal (R_0), improving soil health. Without nitrogen (N) application, irrespective of tillage and crop establishment (TCE) methods, wheat cultivation has a loss of NRs 5282 ha^{-1} with the lowest B:C ratio of 0.87. Therefore, resource conservation technologies viz. ZT and PRB in association with R_R and abundant N application are sustainable and adoptable for wheat cultivation in the terai region of Nepal.

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Seed Treatment with *Trichoderma harzianum*: Suitable Option for Leaf Blast Management of Sub1 and non-Sub1 Rice Genotypes

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ABSTRACT

An experiment was conducted in trays to evaluate the efficacy of seed treatment with different fungal and bacterial isolates against leaf blast, caused by *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), in rice genotypes: Swarna, Swarna Sub1, Samba Mahsuri and Samba Mahsuri Sub1 during 2012 and 2013 at Regional Agricultural Research Station, Tarahara, Sunsari, Nepal. After complete submergence of 15 day old seedlings for five days and one week of recovery, seedlings were transferred in the blast screening nursery where blast pressure was already created. Three scorings were done at 3-day intervals. Isolates varied significantly for leaf blast control of rice over both the years. Rice genotypes also differed significantly for leaf blast development as measured by area under disease progress curve over the years. Environmental factors affected the biocontrol activities of isolates leading to significant interaction between isolates and year. Confounded effect of environmental factors and differential activities of isolates caused genotypes to express differential susceptibility to disease resulting in significant interaction between genotypes and year, and genotypes and isolates during 2012 and for combined analysis, respectively. Among the isolates, IRRI-3 and IRRI-4 isolates of *Trichoderma harzianum* were consistently the most effective in reducing leaf blast at the seedling stage of rice during both the years. Thus, these isolates might be used as seed treatment for leaf blast management. Among the genotypes, Swarna Sub1, with or without seed treatment, recorded the lowest leaf blast severity and could be promoted for cultivation in the flash floods and other rainfed lowland in blast prone areas of Nepal terai.

Key words: *Magnaporthe oryzae*, *Pyricularia oryzae*, rice blast, seed treatment, sub1 rice, non-sub1 rice, *Trichoderma*

INTRODUCTION

Rice in Nepal is a staple food crop and grown in 44% of the total cereal area with 54% of the total cereal production (ABPSD 2012). The crop is the important means of livelihood for the Nepalese people involved directly in rice farming and its related trade and business sectors. It is cultivated in diverse environments covering lowland plain areas to high hills up to 3000 m above sea level (NARC 1997). Plain terai constitutes 73% rice area with 75% of the total production. However, the production and productivity of rice is often negatively affected by several biotic and abiotic stresses (Upadhyay

1996). Present context of climate change has imposed additional difficulties in mitigating such stresses. Almost 15% rice area suffers with flash floods and 30% area is prone to drought. Uncertainty of rainfall and occurrence of flash floods affect plant stand seriously depending on duration of drought and/or submergence and are major threats to higher rice production (RARS 2010). The submergence tolerance gene, *SUB1A,* was identified as the major determinant of submergence tolerance (Xu et al 2006, Septiningsih et al 2008, Bailey-Serres et al 2010, Singh et al 2010). Using marker assisted backcrossing, the *SUB1A* gene was transferred to eight rice varieties (Collard et al 2013). Nepal received the seeds of these varieties from International Rice Research Institute (IRRI) in 2008 and after testing for few years, released Swarna Sub1 and Samba Mahsuri Sub1 for flash floods affected areas in 2011 (Singh et al 2013). The new varieties have a small segment of the donor genome containing *SUB1A*, while retaining the entire genome of the original varieties (Singh et al 2009, Bailey-Serres et al 2010, Iftekharuddaula et al 2011, Sarkar and Bhattacharjee 2011). In Nepal, these Sub1 varieties are gaining popularity among the farming communities even in flash flood non-affected lowland areas.

Among biotic factors, blast, caused by *Magnaporthe oryzae* Couch and Kohn (anamorph *Pyricularia oryzae* Cav.), is the widespread and destructive disease in Nepal (Manandhar 1987, NARC 1997). It damages seedlings in the seedbed (Chaudhary and Sah 1998) and affects plant growth and yields in the transplanted field in terms of quality and quantity (Ou 1980, Manandhar et al 1985, Chaudhary 1999). Rice growers often face shortage of seedlings for transplanting in the areas where blast susceptible but popular varieties are grown (Chaudhary et al 1994, Chaudhary and Sah 1997, Chaudhary et al 2005). Popular varieties become susceptible after a few years of release when cultivated in a large scale (Kiyosawa 1982, Bonman and Mackill 1988, Bonman et al 1992) because the blast pathogen is highly variable for genetic adaptation to cause the disease on newly released popular varieties (Chaudhary et al 2004). Seed treatments (Manandhar 1984, Chaudhary and Sah 1998) and foliar sprays with chemicals (Manandhar et al 1985, Chaudhary 1999) have been recommended to reduce its damage. Use of chemical is not practical for resource-poor farmers, and its meticulous handling and environmental polluting nature. Thus, the rice growers often suffer with crop loss.

Use of microbial bio-control agents has been a successful and eco-friendly means of plant disease control for the last eight decades (Weindling 1934, Weindling and Fawcett 1936). The fungi of the genus *Trichoderma* species (Harman et al 2004, Druzhinina et al 2011, Zaidi and Singh 2013) and rhizobacteria in the genera *Pseudomonas, Bacillus, Streptomyces, Enterobacter* and others (Pieterse et al 2000, 2001) have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity. Effects of bio-control microbes are still more expressed under stress conditions (Harman 2006). In this study, different species of fungi and rhizobacteria were used as seed treatment in controlling leaf blast of rice under hot spot in eastern terai conditions of Nepal. Inferences were drawn from other reports that demonstrated the control of foliar diseases in other crops by induced systemic resistance with some *Trichoderma* strains although their growth was confined to root zone (Ahmed et al 2000, Harman et al 2004, Harman et al 2010, Shoresh et al 2010). Recently, Mastouri et al (2010) showed that seed treatment with *T. harzianum* strain T22 enhanced tolerance to abiotic and biotic stresses (seed and seedling diseases caused by *Pythium ultimum*) in rice. The present work was new initiative considering the microbial isolates as seed treatment for management of rice leaf blast. The study aims at identifying the effective bio-control microbial isolate(s) for leaf blast management and to evaluate the genotypic differences for blast severity at the seedling stage of Sub1 and non-Sub1 rice genotypes.

MATERIALS AND METHODS

The experiment was laid out in a split plot design; microbial isolates as main-plots and rice genotypes as sub-plots with three replications during the 2012 and 2013 wet seasons at Regional Agricultural Research Station Tarahara, Sunsari, Nepal. Rice genotypes were Swarna, Swarna Sub1, Samba Mahsuri and Samba Mahsuri Sub1. Rice seeds were treated with 10 different microbial isolates (Table 1). Suspension of microbial isolate was prepared by adjusting the concentration of 1×10^5 cfu ml^{-1}. Twenty gram of wheat flour was mixed in one liter of water and boiled for 15 minutes. The mixture was filtered using muslin cloth after cooling. One gram powder of each microbial isolate was properly mixed in 100 ml of mixture separately to prepare microbial suspension. One kg of rice seed was treated with such preparation and dried in the shade for 3-4 hours before seeding.

Sprouted seeds were grown in plastic trays @ 100g per m^2. The trays were filled with a mix of farm soil and farm yard manure (3:1) and fertilized with 150:22:0 $N:P_2O_5:K_2O$ ha^{-1}. The trays were watered whenever necessary. A total of 11 trays were used for sowing the seeds each for a separate isolate including control. Two rows of each genotype were seeded and replicated thrice within each tray. The seedlings were submerged for 5 days in a submergence tank 15 days after sowing. The trays were taken out of the tank and kept for 7 days near the tank for recovery of the seedlings. The trays were put in the blast screening nursery where natural epiphytotic conditions were already created following the procedure of Chaudhary and Sah (1998). Natural disease spread was allowed from diseased inoculum plot to the test plots (trays). The trays were watered daily in the evening before sunset if rainfall did not occur. Rain water was drained out of the tray daily in the evening and morning to avoid high soil moisture and favor longer dew period in the seedlings. This was done because moisture stress under upland conditions maintains longer leaf wetness duration (Kahn and Libby 1958, Ou 1980). During 2012, seeding was done on 11th August and terminated on the 18th September. During 2013, seeding was done on 18th September and the final scoring was completed on the 26th October. Monthly meteorological data (temperature, humidity and rainfall) during the rice season and daily meteorological data during the experimental period of both the years were used for relating to disease development data for interpretation of the results.

Scoring was begun on the 7th day after placing the seedlings in the blast nursery using 0-9 scale (IRRI 1996) and continued for thrice at 3 day intervals. Scores were converted into disease severity as:

$$\text{Leaf blast severity (\%)}=\frac{\text{Score recorded}}{9}\times100$$

AUDPC values were calculated as per the procedure of Shanner and Finney (1977) using the following formula:

$$\text{AUDPC}=\sum_{i=1}^{n}\left[\frac{(y_{i+1}+y_i)}{2}\right]\times(x_{i+1}+x_i)$$

Where, y_i = disease severity at the i^{th} observation, x_i = time at the i^{th} observation, and n = total number of observations

Table 1. A list of microbial isolates used for seed treatment for their efficacy in reducing leaf blast on Sub1 and non-Sub1 rice genotypes at RARS Tarahara, Sunsari, Nepal during 2012 and 2013

Isolates	Source	Bio-control Microbes
TH-3	IRRI/India	*Trichoderma harzianum*
IRRI-2 (TNS-2)	IRRI/India	*Trichoderma harzianum*
FP-10	IRRI/India	*Fusarium pallidoroseum*
IRRI-4	IRRI/India	*Trichoderma harzianum*
IRRI-3	IRRI/India	*Trichoderma harzianum*
IRRI-1	IRRI/India	*Trichoderma harzianum*
T. viride	IRRI/India	*Trichoderma viride*
B-14	IRRI/India	*Bacillus thuringiensis*
T. viride	PPD* Nepal	*Trichoderma viride*

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Analysis of variance (ANOVA) was performed using MSTATC to compare the efficacy of microbial isolates and genetic differences for disease development. Based on ANOVA result, Duncan's multiple range test (DMRT) was performed to compare the treatments. Pooled analysis of two years' data was also done to understand the consistency of microbial isolates in managing leaf blast and genotypic differences for disease development over two seasons, and to understand interaction effect between microbial isolates and year (M×Y), rice genotypes and year (G×Y) and rice genotypes and microbial isolates (G×M).

RESULTS AND DISCUSSION

The use of microbial isolates as seed treatment and rice genotypes had significant influence on leaf blast disease at the seedling stage as measured by AUDPC values (Table 2). GxM interaction for AUDPC values was significant during 2012, indicating that genotypes had variation in disease development as treated with different isolates (Table 2). For example, Samba Mahsuri Sub1 recorded the lower AUDPC values at par with that of Swarna Sub1 when treated with IRRI-4 and IRRI-3. However, the same variety obtained the highest AUDPC value when treated with Nepalese isolate of *T. harzianum*. Swarna Sub1 treated with IRRI-4, IRRI-3 and TH-3 (all *T. harzianum*) recorded the lowest AUDPC values and had the lower AUDPC values in all treated plots. *T. harzianum* isolates IRRI-3 and IRRI-4 reduced leaf blast significantly in seedlings of all genotypes compared to those of other isolates.

Table 2. Effect of seed treatment with microbial isolates and four rice varieties on leaf blast development as measured by AUDPC† values at Regional Agricultural Research Station Tarahara, Sunsari, Nepal during the 2012 wet seasons

Microbial Isolates	Swarna Sub1	Swarna	Samba Mahsuri Sub1	Samba Mahsuri	Mean
Trichoderma harzianum-3	185.2 l-q	96.3 q	303.7 d-k	351.9 b-g	234.3 CD
T. harzianum isolate IRRI-2	140.7 pq	244.4 h-p	270.4 f-m	314.8 c-j	242.6 CD
Fusarium pallidoroseum -10	144.4 pq	218.5 j-p	244.4 h-p	285.2 e-l	223.1 CDE
T. harzianum isolate IRRI-4	159.3 o-q	151.9 o-q	163.0 o-q	218.5 j-p	173.1 E
T. harzianum isolate IRRI-3	185.2 l-q	151.9 o-q	192.6 l-q	266.7 f-n	199.1 DE
T. harzianum isolate IRRI-1	233.3 i-p	255.6 g-o	314.8 c-j	318.5 c-j	280.6 BC
T. viride	159.3 o-q	207.4 k-p	374.1 b-e	363.0 b-f	275.9 BC
Bacillus thuringiensis -14	177.8 m-q	225.9 i-p	325.9 c-i	381.5 b-e	277.8 BC
T. viride Nepalese isolate	155.6 o-q	166.7 n-q	340.7 c-h	392.6 a-d	263.9 C
T. harzianum Nepalese isolate	148.1 pq	225.9 i-p	477.8 a	440.7 ab	323.1 AB
Control	240.7 h-p	266.7 f-n	414.8 a-c	444.4ab	341.7 A
Mean	175.4 Z	201.0 Y	311.1 X	343.4 W	

† AUDPC= Area under disease progress curve, values followed by different letters within a column are significantly different at P=0.05

Effect of microbial isolates was significant for AUDPC values on Sub1 and non-Sub1 rice genotypes in both the years (Table 3). AUDPC values varied from 173 to 342 in 2012 and from 294 to 392 in 2013 as influenced by seed treated with different microbial isolates. All the microbial isolates were effective in minimizing leaf blast of rice compared to control except domestic strain of *T. harzianum.* The pooled analysis revealed that the strains IRRI-4 and IRRI-3 (*T. harzianum*) were the most effective biocontrol agent in reducing leaf blast among the tested microbial isolates in both the years.

Table 3. Effect of seed treatment with microbial isolates on leaf blast of rice tested and measured by AUDPC values at Regional Agricultural Research Station, Tarahara, Sunsari, Nepal during the 2012 and 2013 wet seasons

Microbial isolates	AUDPC†		
	2012	2013	Pooled
Trichoderma harzianum-3	234.3 cd	386.2 ab	310.2 cd
T. harzianum isolate IRRI-2	242.6 cd	355.6 bc	299.1 cd
Fusarium pallidoroseum -10	223.1 cde	377.8 abc	300.4 cd
T. harzianum isolate IRRI-4	173.1 e	294.3 d	233.7 e
T. harzianum isolate IRRI-3	199.1 de	315.2 d	257.1 e
T. harzianum isolate IRRI-1	280.6 bc	307.0 d	293.8 d
T. viride	275.9 bc	348.8 c	312.3 cd
Bacillus thuringiensis -14	277.8 bc	376.5 abc	327.1 bc
T.viride Nepalese isolate	263.9 c	352.8 bc	308.3 cd
T. harzianum Nepalese isolate	323.1 ab	384.8 ab	353.9 ab
Control	341.7 a	391.6 a	366.6 a
SE	17.34	10.48	10.13
CV (%)	20.16	6.19	13.04

† AUDPC, Area under disease progress curve. Values followed by different letters within a column are significantly different at P=0.05

Irrespective of seed treated with different isolates, rice genotypes differed significantly for leaf blast in the seedling stage as measured by AUDPC values in both the years (Table 4). The AUDPC values ranged from 175 to 343 among the varieties in 2012 while disease development was higher in 2013 ranging from 314 to 400.0. Swarna Sub1 had the lowest disease development whereas Samba Mahsuri recorded the highest AUDPC values in both the years.

Table 4. Effect of Sub1 and non-Sub1 rice treated with microbial isolates on leaf blast measured by AUDPC values at Regional Agricultural Research Station, Tarahara, Sunsari, Nepal during the 2012 and 2013 wet seasons

Rice Varieties	AUDPC Values†		
	2012	2013	Pooled
Swarna Sub1	175.4 d	314.2 c	244.8 d
Swarna	201.0 c	353.5 b	277.2 c
Samba Mahsuri Sub1	311.1 b	347.0 b	329.1 b
Samba Mahsuri	343.4 a	400.0 a	371.7 a
SE	9.0	3.8	4.9
CV (%)	20.16	6.19	13.04

†AUDPC, Area under disease progress curve. Values followed by different letters within a column are significantly different at P=0.05

Pooled M×Y, G×Y and G×M interactions for disease development measured by AUDPC values were also found significant (Figure 1). In general, disease development was higher in 2013 as compared to 2012 wet season. Swarna Sub1 had the lowest leaf blast severity on seedlings grown from seed treated with all isolates as compared to those of other genotypes except Swarna seedlings grown from seed treated with isolate TH-3 that had the lowest leaf blast in the 2012 season (Figure 1A). Swarna Sub1 had the lower disease even without seed treatment indicating that the genotype possessed higher level of resistance than other genotypes. Samba Mahsuri Sub1 varied for disease severity as treated with different microbial isolates. It recorded higher leaf blast severity when grown without seed treatment and from seed treated with isolate Th-N but showed as lower disease severity as Swarna Sub1 when grown from seed treated with IRRI-4 isolate of *T. harzianum* leading to significant G×M interaction over the 2012 and 2013 seasons (Figure 1A).

Irrespective of rice genotypes, seedlings grown from seeds treated with isolate IRRI-4 showed the lowest disease development followed by isolate IRRI-3 in both the years. Seedlings grown from seed treated with isolate IRRI-1 recorded higher disease severity in 2012, but it was as effective as IRRI-4 and IRRI-3 isolates in minimizing leaf blast in 2013 (Figure 1B). Similarly, isolate TH-3 was more effective in 2012 and less effective in 2013 in reducing leaf blast. Likewise, *Fusarium pallidoroseum* isolate FP-10 was more effective for disease management in 2012 but was less effective in the following year. Because of these reasons, MxY interaction was significant (Figure 1B). Irrespective of microbial isolates used for seed treatment, rice genotype Samba Mahsuri Sub1 had higher leaf blast severity in 2012 and lower in 2013 as compared to Swarna (Figure 1C). This supported why GxY interaction was significant.

The use of bio-control agent for disease management has become the global concern. In our study, isolates of fungi and bacteria were used as seed dressing agents to evaluate their efficacy in reducing leaf blast of Sub1 and non-Sub1 rice genotypes at the seedling stage. Irrespective of microbial isolates used for seed treatment, Swarna Sub1 showed higher level of resistance to leaf blast at the seedling stage during the 2012 and 2013 tests (Table 4). During the 2013 test, GxM interaction was insignificant for leaf blast development while it was significant during the 2012 test and also was significant when combined analysis of two year's data was performed (Figure 1 and Table 2).

Abilities of bio-control agents to increase plant growth and induce resistance to plant stresses had been reported earlier (Harman and Kubicek 1998). Recent analysis using genetic or mutational approaches, biocontrol of *Rhizoctonia solani* on cotton seedlings by *T. virens,* and *Pythium* seed and seedling diseases by *T. harzianum* depicted that control was solely due to induced resistance (Howell et al 2000, Howell 2006, Shoresh et al 2010). Efficient *Trichoderma* strains grew in rhizosphere, colonized vascular systems of plants and established chemical communication leading to changes in plant gene expression due to influence on plant physiology (Shoresh and Harman 2008, Mastouri et al 2010, Shoresh et al 2010). The changes in expression were substantially greater in the shoots than in roots though the fungi were generally confined to the roots (Shoresh et al 2010). Activation of these systems reduced diseases caused by a wide variety of pathogens including fungi, bacteria and even a virus (Harman et al 2004). For example, *T. harzianum* strain T22, added to the soil during transplanting of tomato, reduced late blight disease in tomato by 80% as compared to non-treated plants. Also, seed and seedling disease caused by *Pythium ultimum* in rice was minimized by seed treatment with the same strain of *T. harzianum*. Similar results were obtained in our studies that leaf blast of rice seedlings was mitigated by seed treatment with *T. harzianum* (Table 2 and 3). However, we still need to analyze the plant tissue of the rice seedlings grown from treated and untreated seeds at molecular level to understand the molecular basis for defense mechanism against the blast and to depict host pathogen interaction as affected by *Trichoderma* isolate.

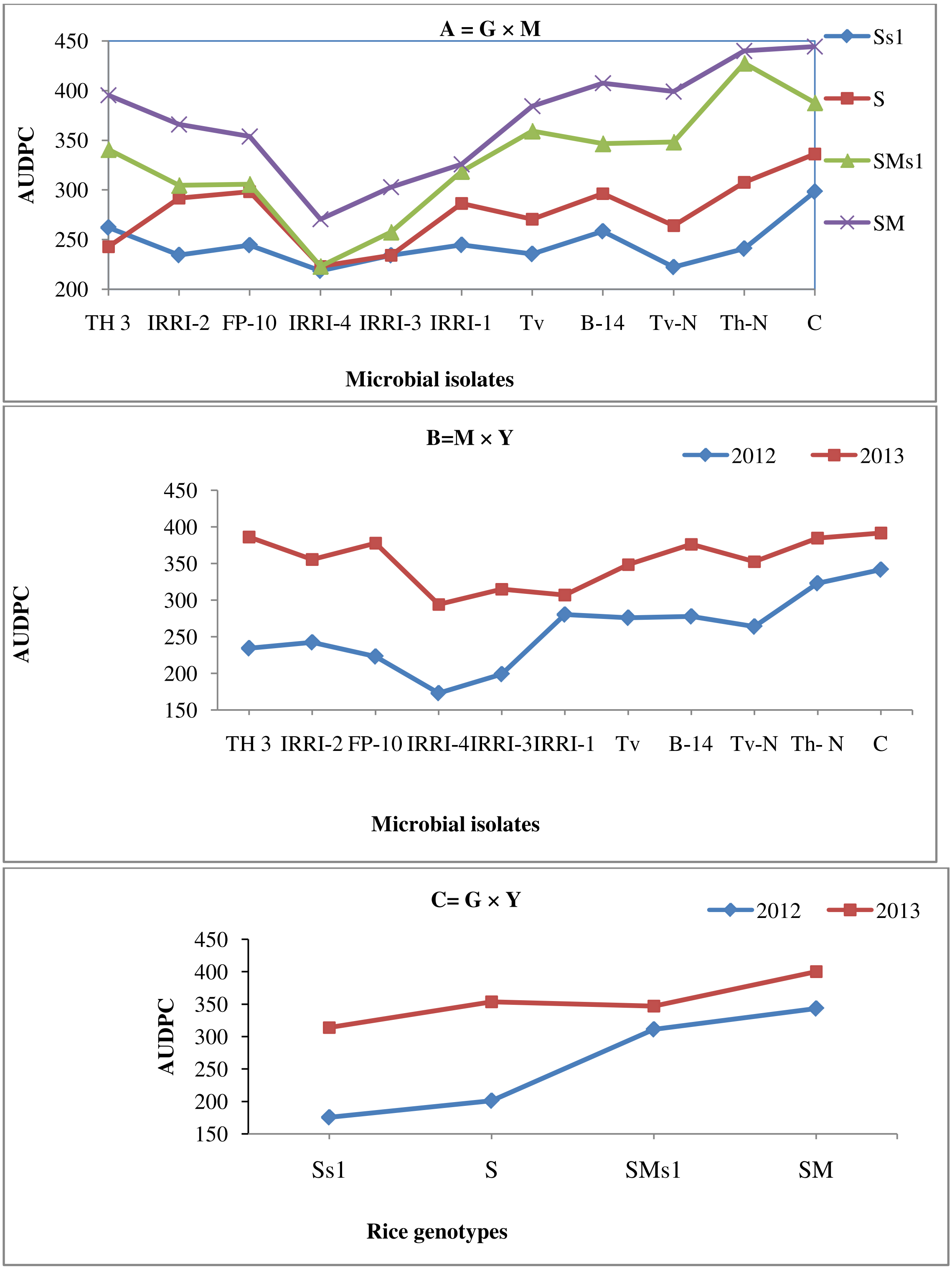

Figure 1. Pooled interaction effect of four rice genotypes (Ss1 = Swarna Sub1, S = Swarna, SMs1 = Samba Mahsuri Sub1 and SM = Samba Mahsuri) and seed treatment with microbial isolates (TH-3 = *Trichoderma harzianum*, IRRI-2 = *T. harzianum*, FP-10 = *Fusarium pallidoroseum*, IRRI-4=*T. harzianum*, IRRI-3= *T. harzianum* , IRRI-1 = *T. harzianum* , Tv = *Trichoderma viride*, B-14 = *Bacillus thuringiensis*, Tv-N = *T. viride* Nepal, Th-N = *T. harzianum* Nepal and C = control) over 2012 and 2013 for rice leaf blast development measured by AUDPC at RARS Tarahara, Nepal.