

Research Article



Effect of Bed Planting and Zero Tillage on Productivity and Water Use of Irrigated Maize –Wheat Cropping System in Khyber Pakhtunkhwa Province of Pakistan

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Abstract | Crop establishment methods like broadcasting of wheat and manual planting of maize on flat surface coupled with flood irrigation are used in irrigated maize–wheat system of Khyber Pakhtunkhwa (KP) province that requires skilled labor, intensive tillage and more water for irrigation. In this study, zero tillage and bed planting effect in comparison with farmer practice on maize–wheat system productivity was evaluated at five sites in district of Nowshera, KP province during 2014–2016. Maize grain yield with bed planting and manual planting on flat surface were non-significantly different. Because of weed infestation, there was 30 percent lower maize grain yield with zero till planted maize in comparison with bed planting and farmer practice. Zero till drill planted wheat after maize crop had higher grain yield in comparison with mechanized bed planting and farmer practice of broadcasting in 2014–15 and 2015–16 wheat season. Because of higher maize grain yield, maize–wheat system productivity was significantly higher with bed planting and farmer practice in comparison with Zero tillage. Bed planting of maize had 20 – 30 percent higher water use efficiency (WUE) in comparison with flat planting of farmer practice in both the years. Overall comparison of maize – wheat cropping system showed that there were 16 and 22 percent water saving with bed planting in comparison with other practice in 2014–15 and 2015–16 cropping cycle, respectively. The study showed that farmer practice of manual planting of maize and broadcasting of wheat can be replaced with bed planting of maize and zero till drill planting of wheat, respectively to save water, labor and improve maize–wheat system productivity and water use efficiency.

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Introduction

Agriculture contributes 30 percent in GDP of Khyber Pakhtunkhwa province of Pakistan. Wheat and maize are major staple food of the KP province. The province of KP is food insecure region as it produced 1.15 million tons wheat annu-

ally against consumption of 2.9 million tons (GOP, 2015). Improvement in maize–wheat productivity at farm level would not only increase food security of farmer but also improve food availability at affordable prices in the KP province of Pakistan (Ahmad and Farooq, 2010). Average wheat grain yield of farmers in Khyber Pakhtunkhwa province is 1807 kg ha⁻¹

(GOP, 2015) that is far lower than average wheat yield of Pakistan (2775 kg ha^{-1}). Maize (*Zea mays* L.) is second important staple food crop after wheat in the province of KP. Maize farmers in KP produced 0.83 million ton of maize from 0.44 million hectare area with an average yield of $1892 \text{ Kg per hectare}$ which is below average yield (4155 kg ha^{-1}) of Pakistan.

In the province, maize – wheat cropping system occupies a total of 0.80 million hectares that is spread in districts of Abbottabad, Battagram, Buner, Charsadda, Dir Lower, Dir Upper, Haripur, Malakand, Mansehra, Mardan, Nowshera, Peshawar and Swabi (GOK, 2014). Maize and wheat productivity in maize – wheat cropping system is low due to traditional planting techniques, inefficient fertilizer, and irrigation management techniques. Planting of maize crop is predominantly manual and farmer practice variety of methods like broadcasting and line sowing with hand planting. Small farmers plant opens pollinated varieties of maize through broadcasting followed by shallow cultivation; this method leads to wastage of seed and non-uniform crop stand. Maize hybrid and open pollinated maize varieties are also line planted that involve placing of rope or string with mark on a specific distance in lengthwise and maize seed is planted with hoe, the method is known as Thapa method. This method is labor intensive and can only be adopted with availability of skilled labor. In other method, furrow is made with the help of hand plough and farmers place the seed at approximate distance and seed is covered with planking. These planting techniques are used in maize planting on flat surface and require experienced labor. After maize harvesting, farmers tend to plant wheat through broadcasting of seed and fertilizer with two to three tillage operation that result in poor crop stand and reduced fertilizer use efficiency.

Conservation agriculture techniques including zero till and bed planting reduce operational costs that include machinery, labor, and fuel (Jat et al., 2014) while increasing yields and better utilizing natural resources (Roy et al., 2009). Bed planting (BP) and zero tillage (ZT) combined with residue retention have the potential to improve soil health, reduce production cost, and increase productivity in maize and wheat (Govaerts et al., 2006). However, (Cameron et al., 2015) in a meta - analysis of more than 600 sites reported that there could be yield reduction with no-till and negative impact of no-till were smallest

for wheat (-2.6%) and largest for rice (-7.5%) and maize (-7.6%). Crop yields in the first 1–2 years following no-till implementation declined for all crops except oilseeds and cotton, but matched conventional tillage yields after 3–10 years. Planting of crops on permanent beds increased soil available water capacity, improved water infiltration, and aggregate stability in comparison with conventional tilled crops without beds (McHugh et al., 2009; Verhulst et al., 2011). Wheat yields by adopting zero tillage or reduced tillage are either equal or even better than those obtained with conventional practices because of timely planting of wheat, efficient use of fertilizers and weed control.

Maize planted on flat surface required more water in comparison with raised bed and furrow irrigation system. Bed and furrow planting systems improved water distribution (Hobbs et al., 1998), reduced irrigation water requirement and improved water use efficiency in rice-wheat cropping system of Indo-Gangetic plain (Connor et al., 2003; Choudhury et al., 2007) and provides drainage in water logged fields (Sayer and Ramos, 1997). Raised bed planting allows better stand, uses less seed rate, provides opportunity for mechanical weed control, permits band application of fertilizers, reduces crop lodging (Govaerts et al., 2006; Wang et al., 2014). Bed planting of 4 lines on 90 cm wide beds had 15 percent higher wheat yield and nitrogen use efficiency in comparison with flat planting (Majid et al., 2015).

Raised bed planting system saves 30-40% irrigation water followed by fewer weeds, reduced lodging that help to maintain better crop stand and improved crop productivity (Mann and Meisner, 2015). Permanent raised beds have also been an effective way to improve water management, enhance crop growth and increase crop yield (Sayer, 2004; Singh, 2003). Raised bed planting system is also reported with significantly increasing grain yield due to improved air-filled porosity and plant available water capacity (Bakker et al., 2005; Holland et al., 2007). In comparison with conventional flat planting, raised bed planting system required 30% less irrigation water, with 20% increased WUE (Wang et al., 2004; Ahmad et al., 2014). Good crop yield in raised bed plantings is correlated to higher fertilizer use efficiencies and improvement in root growth. Water sensitive crops like maize and legumes must be grown under good water drainage system. A good irrigation water and drainage system is thus provided by permanent raised beds (Singh et

al., 2008). Zero tillage practice is also reported with improved WUE and soil health over many soil types, cropping systems and climates as well as sustaining productivity of soil and protecting environment (Hobbs, 2007).

Conservation tillage practices like zero tillage and minimum tillage with residue on flat and raised beds improve soil environment, improve WUE and productivity, therefore, conservation tillage approach is a must for practicing sustainable and climate smart agriculture in the sub-mountainous region. The study was conducted at farmer fields with the objectives to evaluate effect of planting techniques such as; zero tillage and raised bed planting on productivity of irrigated maize-wheat cropping system in district of Nowshera in the province of Khyber Pakhtunkhwa.

Materials and Methods

This study was conducted at five sites located in Pirsabaq village of district Nowshera, Khyber Pakhtunkhwa province in Pakistan (Figure 1). The village Pirsabaq is located on the bank of river Kabul (34° 01' N, 72° 02' E longitude and 288 m above sea level). Three out of five sites were farmer fields and other two included fields located at Cereal Crops Research Institute (CCRI) and Agriculture Extension Farm. Soil type of experimental sites were silty loam having 0.7-0.9 percent organic matter, pH of 7.7, 4.5 – 6.0 mg available phosphorus / kg Olson- P and 100 mg available K / kg of soil. Before initiation of the study, the sites were under irrigated maize-wheat cropping system for more than five years. Average annual rainfall in Nowshera is 532 mm. Amount of rainfall and its distribution for maize 2015 and 2016 and wheat 2014-15 and 2015-16 is presented in Figure 2 and 3.

After harvesting of wheat crop, planting techniques plots were established in June 2014 and first autumn maize crop 2014 was considered as zero crop. In this study, three planting techniques such as; Farmer practice, raised bed planting and Zero tillage were evaluated on 5 replications (sites) in a Randomized Complete Block Design in a maize-wheat rotation during 2014-15 and 2015-16. At each site, three planting techniques were established and each experimental unit consisted of 1333 m² plot. The details of various field operations followed in planting techniques were as under.

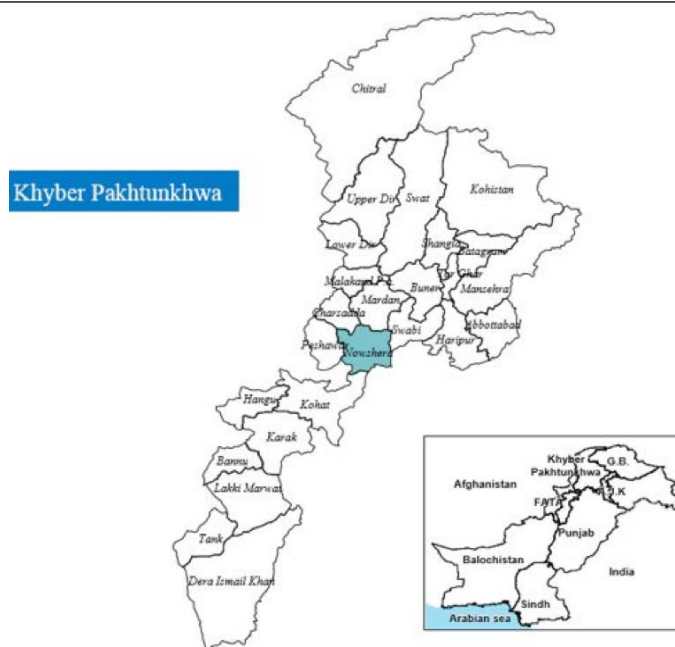


Figure 1: Map showing district Nowshera in Khyber Pakhtunkhwa province of Pakistan.

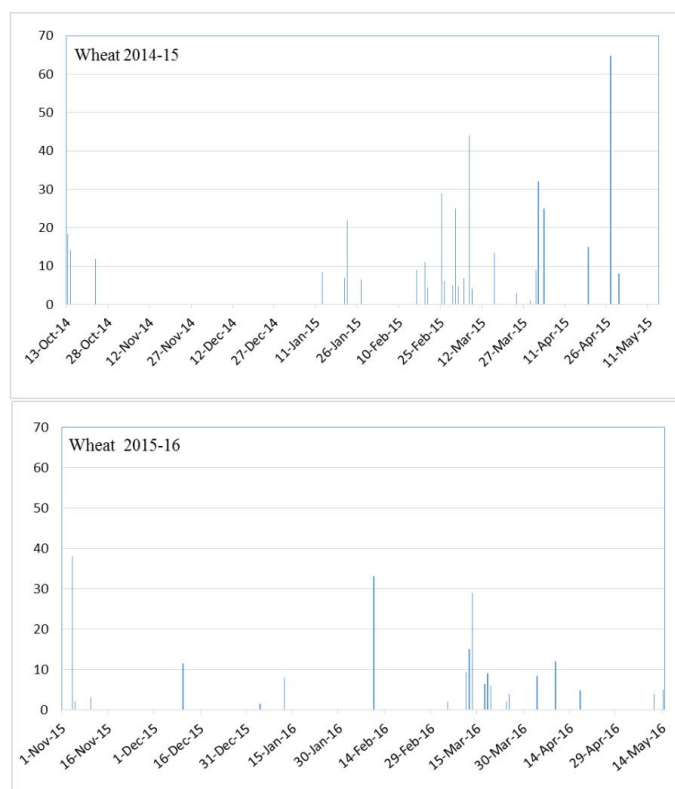


Figure 2: Rainfall received during wheat season 2014-15 and 2015-16 at CCRI, Nowshera, KP.

For wheat planting with farmer practice, maize was hand harvested leaving residue at 15 cm height from soil surface and soaking irrigation was applied. Land was prepared with two plowings followed by broadcasting diammonium phosphate fertilizer (DAP) and wheat seed. Afterwards, shallow cultivation followed by levelling with wooden plank was performed for mixing fertilizer and wheat seed in to soil.

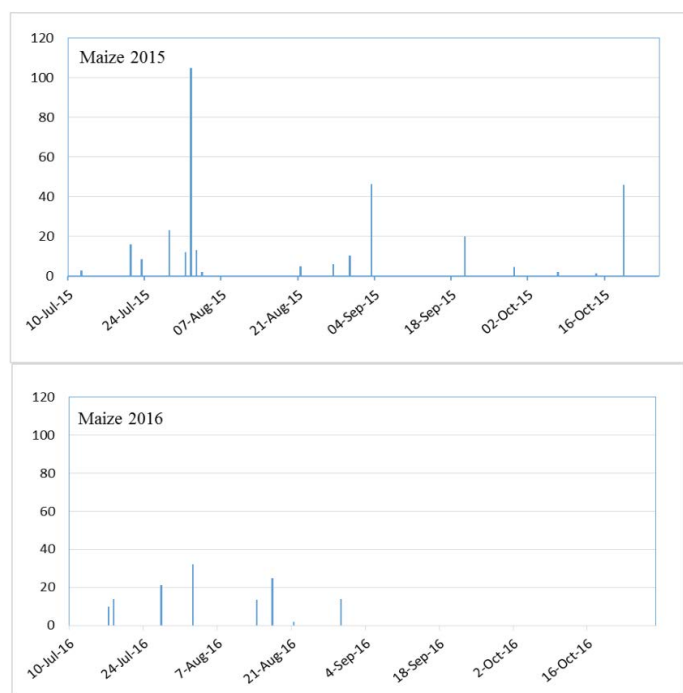


Figure 3: Rainfall received during maize season 2015 and 2016 at CCRI, Nowshera, KP.

For maize planting with farmer practice, wheat was hand harvested at 15 cm residue height from soil surface and soaking irrigation was applied. Fields were plowed two times with cultivator followed by broadcasting of DAP fertilizer. Afterwards, shallow cultivation followed by levelling with wooden plank was performed for mixing of fertilizer in to soil. Planting rows were marked with marker maintaining row to row distance of 70 cm and maize was planted manually with 20 cm plant to plant distance.

For wheat bed planting, maize crop was harvested at 30 cm height and soaking irrigation of 60 mm was applied. Herbicide Roundup @ 2500 ml per hectare in optimum moisture condition was applied for weeds control. DAP fertilizer and wheat seed was placed in on permanent raised beds with the help of National Multicrop Zero till bed planter manufactured by National Agro Industries, Ludhiana, India in standing maize residue. National Multicrop Zero till bed planter shape beds, apply fertilizer and seed in one operation. Width of each bed was 70 cm and two rows of wheat planted on flat top of each bed.

For maize bed planting, wheat crop was harvested at 15 cm height and soaking irrigation of 60 mm was applied and weeds were controlled with herbicide Roundup. DAP fertilizer and maize seed was placed on permanent raised beds with the help of National Multicrop Zero till bed planter in standing wheat

residue. Width of each bed was 70 cm and one row of maize planted on center of flat top bed with plant to plant distance of 20 cm.

For wheat planting under zero till conditions, maize plot was hand harvested at 30 cm height and soaking irrigation was applied. Herbicide Roundup was applied to control weeds. DAP fertilizer and wheat seed was placed with National Multicrop Zero till planter manufactured by National Agro Industries, Ludhiana, India in standing residue with row to row distance of 23 cm.

For maize planting under zero till condition, wheat crop was harvested at 15 cm height and pre-sowing irrigation was applied. Afterwards, herbicide Roundup was applied to control weeds. DAP fertilizer and maize seed was placed with National Multicrop Zero till planter in standing wheat residue. In zero till planting, row to row distance and plant to plant distance for maize crop was 70 and 20 cm, respectively.

Wheat variety Pirsabak – 2013 was seeded with seed rate of 125 kg/ha on farmer practice and zero tillage plots on November 9 and 19 during 2014 and 2015, respectively. However, seed rate of 100 kg /ha was used in raised bed planting of wheat on November 9 and 19 during 2014 and 2015, respectively. Maize hybrid P-30k08 (Pioneer) was seeded with seed rate of 20 kg / ha in farmer practice, raised bed planting and zero till plots in Pirsabaq, Nowshera on July 13 and 17 in 2015 and 2016, respectively.

All planting techniques plots received 137 kg of N and 57.5 kg of P per hectare in maize and wheat. All 57.5 kg phosphorus and 22.5 kg N per hectare in the form of diammonium phosphate was broadcasted and mixed with plowing in farmer practice plots. However, DAP fertilizer was drilled along with seed at planting in raised bed planting and zero till planting techniques. Remaining 115 kg N per hectare in the form of urea fertilizer was applied in two equal doses at first and 2nd irrigation in wheat crop. However, 115 kg N per hectare in the form of urea was applied in two doses at V6 (25-30 days after planting) and V10 (35-40 days after planting) in maize crop.

Weeds that germinated before planting of wheat and maize were controlled with tillage in farmer practice. However, herbicide Roundup @ 2500 ml per hectare was applied on zero tillage and raised bed plots be-

fore planting to control weeds that germinated before planting of maize or wheat. In maize, herbicide (Primextra @ 2000 ml / ha) was applied immediately after planting. In wheat crop, herbicide (Affinity @ 2000 ml /ha) was applied 25-30 days after crop emergence to control narrow and broadleaf weeds.

Irrigation management for these sites included irrigation water from watercourse and rainfall. In wheat crop, soaking irrigation of 60 mm was applied in both year 2014-15 and 2015-16 before wheat planting. In Nowshera district of Khyber Pakhtunkhwa province, canal closure starts from January 10, so farmers applied two irrigations each of 60 mm that coincided with nitrogen application in the form of urea before January 10 at tillering and jointing stages. After January 10, water requirement for wheat crop was met through rainfall during booting and grain filling period. In case of maize crop, farmers received water from canal every week and a total of 11 and 12 irrigations including soaking irrigation were applied in 2015 and 2016, respectively. The total amount of water applied was computed as the sum of irrigation water applied and received from rainfall. The water use efficiency (WUE) was calculated with following formula:

$$WUE = \text{grain yield (kg/ha)} / \text{Total water applied (mm / ha)}$$

Soil samples were collected at the start of study from 0-15 and 15-30 cm depth using an auger. Each sample was composited from three points in a field. These samples were air-dried passed through a 2 mm sieve and were analyzed for Olsen-P (Knudsen et al., 1982) and NH₄OAc – extractable K using the method described by Olsen and Sommers (1982). Soil organic C was also analyzed by Walkley and Black method described by Olsen and Sommers (1982).

All the data on grain yield and its associated parameters of wheat and maize were analyzed with Statistix 8.1 using year and planting technique as factors. Tukey Test / LSD was used at the P< 0.05 level of probability to test the differences between treatment means.

Results and Discussion

Maize crop

Different planting techniques had significant effect on grain yields during 2015 and 2106 autumn maize crop. Maize grain yields with mechanized bed

planting and farmer practice of manual planting on flat land were non-significantly different during both years (Table 1). Whereas maize grain yields with zero till planted maize were lower in comparison with bed planting and manual planting techniques. There was 30 percent reduction in grain yield of zero till planted maize in comparison with bed planting and farmer practice in both years that was mainly attributed to higher weed infestation in zero till planted maize (Cameron et al., 2015). Because of rainfall in early July, higher weed infestations in zero till maize plots at planting was noted that reduced herbicides effect and resulted in reduced plant population in zero till in comparison with other planting techniques of bed planting and farmer practice (Table 2). Weed infestation in zero till plots affected initial maize growth and contributed towards reduced cob length and number of grains per cob that ultimately reduced maize grain yield (Table 2).

Table 1: Grain yields of wheat and maize under various planting techniques in maize – wheat system.

Planting technique	Wheat		Maize			
	2015	2016	Mean	2015	2016	Mean
Bed planting	3007 b*	2747 ab	2877 b	3991 a	4617 a	4304 a
Zero till	3978 a	3080 a	3529 a	2231 b	3790 b	3011 b
Farmer practice	3368 ab	2595 b	2981 b	3967 a	4707 a	4337 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level of probability by LSD test.

Better grain yield of maize with mechanized bed planting and farmer practice were attributed to better plant population and uniform plant to plant distance (Govaerts et al., 2006; Wang et al., 2009). Furthermore, furrow shovels of bed planter uprooted weeds in the furrows and reshaped beds at maize planting in wheat crop residue. Zero till seed openers of multicrop bed planter also partially tilled beds and uprooted any weeds that were left after pre-planting herbicide application on these permanent beds. Better maize population with more uniform plant to plant distance in bed planted maize had a positive effect on cob length, number of grains in a cob and grain size that contributed towards better maize grain yield. This showed that mechanized bed planting of maize could be adopted by farmers in irrigated maize wheat cropping system in Khyber Pakhtunkhwa province of Pakistan.

Wheat crop

Two years mean grain yield mean showed that different

Table 2: Yield components of maize under various planting techniques in maize – wheat system.

Planting technique	No of plants / ha		Grains /Cob (No)		Spike length (cm)		1000 - grain wt. (g)	
	2015	2016	2015	2016	2015	2016	2015	2016
Bed planting	43100 b*	53300 a	404 a	361 ab	15.4 a	20.2 a	318 a	291 b
Zero tillage	33500 c	48200 b	330 b	317 b	11.2 b	17.0 b	270 b	274 b
Farmer practice	47800 ab	51600 a	395 a	393 a	14.8 a	20.6 a	305 ab	342 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level of probability by LSD test.

planting techniques significantly affected wheat grain yield in this planting techniques study. Zero till drill planted wheat had higher grain yield in comparison with mechanized bed planting and farmer practice of broadcasting in both wheat seasons (Table 1). However, differences in wheat grain yield with zero tillage drill and farmer practice of broadcasted wheat in 2014-15 were statistically non-significant. Lower wheat grain yield with mechanized bed planting in comparison with other planting techniques was attributed to significantly lower plant population, reduced tillers and fertile spikes per unit area in comparison with zero till planted wheat (Table 3). In zero till planted plots, wheat seed was drilled in 23 cm apart rows. Whereas in bed planting, two rows of wheat were planted on top of each bed with 70 cm total bed and furrow width that resulted in row to row distance of 35 cm. This reduction in number of rows per unit area resulted in significant reduction of wheat crop stand at planting and contributed towards lower yield of bed planted wheat. In Zero till planted wheat, plant population was at par with broadcasted wheat and better than bed planted wheat. However, row to row distance of 23 cm contributed towards higher plant population, better tillering and wheat growth in zero till wheat in comparison with bed planted and farmer practice of wheat planting (Goovaerts et al, 2006). Planting techniques did not have significant effect on spike length in both years (Table 3). However, thousand grain weight was significantly higher in bed planted wheat in both years in comparison with other planting techniques.

Maize – wheat system

Because of higher maize grain yields, maize –wheat system productivity was significantly higher with bed planting and farmer practice in comparison with zero tillage in both cropping system cycles (Table 4). Bed planting system had advantage of saving in cost of land preparation in the tune of Rs. 15000 per hectare in cropping cycle over farmer practice that helped to increase farmer profit. Better wheat grain yield with zero till technique could not compensate the effect of

reduced maize yield in maize – wheat system productivity. Maize and wheat productivity was higher with bed planting and zero tillage planting technique, respectively (Table 1). This finding showed that farmers have choice and they can use multicrop bed planter for maize planting after land preparation and wheat can be mechanized planted with zero tillage wheat drill.

Table 3: Yield components of wheat under various planting techniques in maize – wheat system.

Planting technique	Emergence (plant m ⁻²)		Tillers m ⁻²		Spike length (cm)		1000 grain wt. (g)	
	2015	2016	2015	2016	2015	2016	2015	2016
Bed planting	136 b*	121 b	245 b	247 b	11.3 a	11.0 a	41 a	46 a
Zero tillage	184 a	167 a	297 a	270 a	10.6 a	10.7 a	41 a	43 b
Farmer practice	160 ab	181 a	224 b	262 a	10.8 a	10.6 a	39 b	43 b

* Means within a column followed by the same letter are not significantly different at the 0.05 level of probability by LSD test.

Table 4: Grain yield (Kg / ha) of maize – wheat system with planting techniques.

Planting technique	2014-15	2015-16	Mean
Bed planting	6998 a*	7364 a	7181 a
Zero till	6209 b	6870 b	6540 b
Farmer practice	7335 a	7302 a	7318 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level of probability by LSD test.

Irrigation and water use efficiency (WUE)

Total water application including rainfall received during 2015 maize crop was 13 percent higher in comparison with 2016 because of less rainfall in earlier part of growing period and no rainfall during the month of September 2016 (Figure 1). Maize crop received 11 irrigations in 2015 in comparison with 12 in 2016 season. Total amount of water applied including rainfall was 39 percent higher in 2015-16 wheat in comparison with 2014-15 that was due to 320 mm more rainfall in months of February, March and April 2016 (Figure 2).

Table 5: Total water (mm) including rainfall applied in maize wheat under various planting techniques.

Planting technique	Wheat		Maize		Maize – wheat	
	2015	2016	2015	2016	2014-15	2015-16
Bed planting	534	297	812	662	1346	959
Zero till	588	351	1010	878	1598	1229
Farmer practice	588	351	1010	878	1598	1229

Table 6: Water use efficiency (kg of grains / mm water) in maize wheat under various planting techniques.

Planting technique	Wheat		Maize		Maize – wheat	
	2015	2016	2015	2016	2014-15	2015-16
Bed planting	5.63	9.25	4.92	6.97	10.55	16.22
Zero till	6.77	8.77	2.21	4.32	8.97	13.09
Farmer practice	5.86	7.39	3.93	5.36	9.79	12.75

Wheat crop received 588 and 351 mm water including rainfall and three irrigations during 2015 and 2016 wheat growing season respectively in broadcasted and zero till planting technique (Table 5). However, bed planted wheat received 534 and 297 mm irrigation water including seasonal rainfall that was 9 and 15 percent lower than zero tillage and farmer practice wheat (Table 5). In autumn maize crop, 1010 and 878 mm water including rainfall was applied in both zero till and farmer practice in 2014 and 2015 maize crop, respectively. However, bed planted maize received 812 and 662 mm total water including rainfall that was 20 and 25 percent less in comparison with flat planting of zero tillage and farmer practice (Mann and Meisner, 2003). Overall comparison of cropping cycle results showed that there was 16 percent and 22 percent water saving with bed planting in comparison with other practice in 2014-15 and 2015-16 cropping cycle, respectively (Table 5). In irrigated maize wheat system, 369 mm more water was available during 2014-15 cycle in comparison with 2015-16 that was mainly due to higher rainfall in August 2015 for maize and in February to April 2015 for wheat season that contributed towards higher maize and wheat yield in 2014-15 cycle.

Water use efficiency (WUE) with raised bed planting system was higher in comparison with farmer practice and zero tillage system in both cropping cycle of 2014-15 and 2015-16 (Table 6). In furrow irrigation raised bed planting technique water moves in furrow, so less amount of water is required to irrigate field. In bed planting technique, 10.55 and 16.22 kg of grains

were produced with one mm of water that was 17 - 24 percent higher than zero tillage and 8 - 27 percent higher than farmer practice (Table 6). Water use efficiency of maize – wheat system was higher in 2015-16 than 2014-15 system cycle. More improvement in WUE was observed under low water availability condition of 2015-16 crop cycle. Bed planting technique in maize had 20 - 30 percent higher WUE in comparison with flat planting of farmer practice in both years of 2015 and 2016. In case of wheat, WUE was 15-19 percent higher with zero tillage in comparison with farmer practice of broadcasting wheat in both 2014-15 and 2015-16 crop season. Bed planting in maize – wheat system had higher water use efficiency in comparison with planting techniques on flat surface of farmer practice and Zero tillage. There was water saving of 9-15 percent in wheat and 20-25 percent in maize with bed planting technique that resulted in 25-30 percent in improvement of WUE of bed planted maize – wheat system (Wang et al., 2004 ; Ahmad et al., 2011).

Conclusions

Manual maize planting on flat surface not only require trained labor for planting but also utilize more water. In the wake of emerging water shortage, increasing pumping cost, and labor cost, modification in planting technique would be needed that could help in improving productivity of these low input maize – wheat system with efficient resource utilization. This two-year planting techniques study in irrigated maize-wheat system of Khyber Pakhtunkhwa province showed that farmers can achieve better or at par maize yield with 20-25 percent water saving and ease in irrigation application with bed planting in comparison with farmer practice of manual planting on flat surface. The technique would help farmers to improve WUE in the range of 20-30 percent and this extra water could be utilized for the irrigation of more area. Bed planting adoption during early years in maize – wheat system could maintain system productivity and reduce tillage cost around Rs. 7500 per hectare that would help farmers to improve profits. Farmers of maize - wheat system could improve productivity through adoption of mechanized bed planting for maize and mechanized wheat planting with zero till drill under tilled and no till conditions. Because of weed infestation, zero till maize had lower yield and was not recommended.

Under water shortage scenario, farmers may adopt bed planting in maize – wheat system and that would not only help improve water use efficiency of the cropping system but also increase farmer profit through reduction in cost of land preparation in permanent bed planting.

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Author's Contributions

Imtiaz Hussain and Muhammad Imtiaz conceived and designed the trial. Ibni Amin Khalil, Subhanulla, Gulzar Ahmed, Ansaar Ahmed and Imtiaz Ahmed performed experiments and collected data. Ansaar Ahmed, Gulzar Ahmed and Imtiaz Hussain analyzed the data. Ansaar Ahmed, Imtiaz Hussain and Muhammad Imtiaz wrote the paper.

Conflict of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript and in the decision to publish the results.

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