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Full Length Article

Optimal Supply of Water and Nitrogen Improves Grain Yield, Water Use Efficiency and Crop Nitrogen Recovery in Wheat

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Abstract

Little information is available on the role of optimized application of irrigation and N on crop N recovery and NO_3-N build up and movement in soil profile. A field experiment was carried out to evaluate the effects of irrigation and N management practices on wheat yield, water and fertilizer use efficiency and NO_3-N distribution in soil. The treatments included were three levels of irrigation; 0.7, 1.0 and 1.3% of the estimated evapo-transpiration (ET_c) and four levels of N; 0, 110, 160 and 210 kg N ha^{-1} in split plot design. The N was applied either in two splits (50% at sowing + 50% at maximum tillering) or three splits (50% at sowing + 25% at maximum tillering + 25% at spike initiation). Nitrogen applied at 110 kg ha^{-1} in three splits produced higher wheat yield, N recovery and water use efficiency (WUE) than two splits. Further, application of N in three splits had considerably lesser accumulation of NO_3-N in soil as compared to two splits. A significant irrigation effect was observed on grain yield, N recovery and WUE. The highest levels were achieved with water application according to crop water requirement (1.0 ET_c). The deficit irrigation produced significantly lower grain yield (3.15 t ha^{-1}) than full (3.80 t ha^{-1}) and excessive (3.80 t ha^{-1}) irrigations. Response of WUE to irrigation levels was in the order, 1.0 ET_c (11.03 kg ha^{-1} mm^{-1})>0.7 ET_c (10.63 kg ha^{-1} mm^{-1})>1.7 ET_c (9.84 kg ha^{-1} mm^{-1}). Deficit irrigation resulted in higher build up of NO_3-N in surface soil. In contrast, excessive irrigation resulted in greater concentration of NO_3-N in lower depths of soil. The results from this research show that there is great potential for decreasing N leaching and increasing wheat crop yield and N use efficiency thorugh controlled irrigation and N application according to crop demand.

Keywords: Deficit irrigation; Photosynthesis; Estimated evapotranspiration; Drainage losses; Nitrate leaching

Introduction

Nitrogen being major essential nutrient plays an important role for growth and development of plants. Plants can absorb N both in the form NO_3-N and NH_4-N. Mobility of positively charged NH_4^+ ion in soil is low especially in soils of the temperate regions including Pakistan with alkaline pH. The NO_3-N is highly mobile in soil and can easily leach down the soil profile (Sahrawat, 1982). Sepaskhah and Hosseini (2008) indicated that there was substantial increase in yields of crops including wheat with the application of N. Contrary to this, it has been reported that the yield response to N fertilizer application was not linear (Cossey *et al*., 2002), resulting in very low nitrogen use efficiency (NUE). Low NUE is a major problem associated with many conventional farming systems in the world and results in higher production costs, leading to lower net returns for farmers (Wang *et al*., 2010).

There are number of factors that can affect the build up and movement of residual NO_3-N in soil. Among these, fertilizer and irrigation practices are the most important to consider in order to decrease leaching losses and improve economic yield and environmental sustainability. Research indicates a positive relationship between amounts of N versus leaching of NO_3 away from active root zone (Jalali, 2005). Fan *et al*. (2010) reported very low leaching losses of NO_3-N when application rate was below 150 kg N ha^{-1}, but leaching of NO_3 increased when N rate increased to 225–300 kg ha^{-1}. Therefore, N applied at optimum rate can help minimize leaching losses of NO_3.

A common practice for N application in cereals by farmers in Pakistan is to apply one half (1/2) at sowing and remaining half in two or three equal splits at critical growth stages (FAO, 2004). However, there is no systematic study on the effect of N split application on NO_3 leaching losses. It has been observed that the presence of high N concentration in soil when there is no crop or with low crop's demand viz. before emergence and/or at harvesting, results in low NUE and high leaching losses (Bhardwaj *et al*., 2010; Shi *et al*., 2012). Therefore, the timing and method of fertilizer N application is another

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important factor affecting NO_3 leaching losses, which can be decreased by providing N at the time of maximum uptake (Oberle and Keeney, 1990). Split application of N could improve NUE and reduce NO_3-N leaching losses (Jia *et al*., 2014).

In arid to semi-arid regions, farmers normally apply water more than the crop needs to get optimal yields and to maintain salinity at acceptable levels (Sepaskhah and Hosseini, 2012). However, irrigation water applied at levels exceeding crop needs through conventional irrigation methods is among the major reasons for enhanced N leaching losses (Meisinger and Delgado, 2002). Mirjat *et al*. (2008) conducted field experiments in Sindh, Pakistan to assess the impact of four irrigation methods on NO_3-N movement in soils. The four irrigation methods investigated were two traditional flooding (basin and furrow) and two micro irrigation (trickle and sprinklers) methods. The studies showed that basin and furrow irrigation resulted in leaching of NO_3-N to deeper soil depths than with trickle and sprinkler methods. It was further concluded that concentration of NO_3-N at 1.2 m soil depth as a result of traditional flooding method of irrigation was above the established by National Standards for Drinking Water Quality (NSDWQ, 2008).

Farmers in Pakistan normally apply higher than optimum levels of N and irrigation water, which could result in environmental problems. In the past, little attention has been paid to NO_3-N leaching. Although these regions have less total rainfall all the year round but about 60–70% of the annual precipitation is usually concentrated in monsoon season (July–September). Heavy rainfall in monsoon season transports surface NO_3 deep into the soil profile. This phenomenon is more prevalent in areas where summer fallow procedure is practiced. In addition to heavy rains, the usual flood irrigation which is common farming practice could also cause NO_3 transport to deeper soil layers. In some parts of the country underground waters contained NO_3^- at levels exceeding the maximum permissible concentration suggested by WHO (Imtiaz *et al*., 2004; Kazmi and Khan, 2005; Tahir and Rasheed, 2008). These reports has begun the debate if intensive agricultural activities like high rates of N fertilizers especially at sowing, repeated application of organic manures and/or high levels of irrigation are responsible for high levels of NO_3 in water. Recent literature shows that NO_3 leaching is a global issue. This may or may not be true about Pakistan particularly in Punjab because of the shortage of systematic studies on NO_3-N dynamics in soil-plant system in this region. Therefore, it is need of time to carry out field studies to identify the water saving irrigation practices along with proper rate and time of N application to improve the yield as well as decrease leaching losses of N. This experiment was, therefore, carried out with the objectives to monitor the individual and combined effects of irrigation levels and nitrogen rates and application timing on yield of wheat crop, crop N recovery and NO_3-N distribution in soil.

Materials and Methods

Experimental Site and Climate

A field experiment was conducted at research farm, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad (Latitude 31^0–$26^{'}$ N, Longitude 73^0–$06^{'}$ E and Altitude 184.4 m) during 2011–2013. The climate of the region is subtropical, semi-arid with severe summers and winters (Table 1). Soil of the experimental area is well drained, poor in N and P contents and calcareous comprising of alluvial deposits mixed with loess (Table 2). It belongs to Hafizabad soil series (aridisoil-loam, mixed, semi-active, isohyperthermic Typic Calciargids) in USDA classification.

Treatments and Experimental Design

The experimental site had been following wheat-maize rotation for almost past six years. The experimental lay out was split plot in randomized complete block with three replications. The irrigation regimes and N treatments were randomly allocated to main plots and subplots, respectivley. The plot area was 63 m^2 (7.38 m × 8.53 m). Three rates of N (110, 160 and 210 kg ha^{-1}) were applied as urea (46% N) in either two or three splits. For two equal splits (N_{50} + N_{50}), fertilizer was applied at sowing and tillering stages of wheat. For three split (N_{50} + N_{25} + N_{25}), N was applied at sowing, tillering and spike initiation. Including control (no N application), this constitute seven treatments of N (Table 3). The crop was exposed to three irrigation regimes: (1) $I_{0.7}$ (deficit irrigation) in which crop was watered to compensate 30% less than its evapotranspiration water loss (ET_c) during the previous days after last irrigation, (2) $I_{1.0}$ (full irrigation) in which crop was irrigated to compensate the full evapotranspiration water loss (ET_c) during the previous days after the last irrigation and (3) $I_{1.3}$ (excessive irrigation) in which crop was watered, i.e. 30% more than evapotranspiration water loss during the previous days after the last irrigation. Irrigation levels were chosen to simulate deficit irrigation commonly due to water scarcity situation/unavailability of water to farmers, full irrigation to produce optimum yield, and excessive irrigation to simulate monsoon situations or to study leaching behavior of nitrate. A weather station, which is 300 m far from the experimental site provided input into the ET model (CROPWAT 8.0). The total depth of irrigation water applied to wheat for the above three irrigation treatments was 156.6, 223.7 and 290.8 mm for deficit, full and excessive irrigation, respectively. In case of rainfall received more than the designed irrigation treatments, the scheduled irrigation was skipped.

Crop Management

Seeds of wheat cultivar Sahar-2006 were collected from Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Initial moisture and germination percentages were 10 and 93%, respectively.

Crop was supplied with phosphorus at 85 and 140 kg P_2O_5 ha^{-1} and potassium at 62 and 110 kg K_2O ha^{-1}. Single super phosphate (SSP) and sulfate of potash (SOP) were used as sources of phosphorus and potassium, respectively. Crop was sown with a seed rate of 150 kg ha^{-1}on December 10, 2011 and was harvested on April 21, 2012. Flat sowing of crop was achieved by planting with hand drill at 22 cm row interval. Recommended agricultural practices were carried out during the study period. The plots were ensured weed free manually and/or by application of weedicides (Atrazine-38SC). Likewise, crop was kept free from insect and pathogen attack by pesticide application.

Irrigation Scheduling According to Estimated Evapotranspiration

The ET_0 (reference crop evapotranspiration) was calculated by computer softawre CROPWAT 8.0 that calculates the ET_0 using Penman-Monteith FAO-56 Equation (Allen *et al*., 1998).

$$ET_0 = \frac{0.408\,\Delta\,(Rn - G) + \gamma \frac{900}{Tmean + 273}\mu 2\,(es - ea)}{\Delta + \gamma\,(1 + 0.34\,\mu_2)}$$

Where, Rn stands for net radiation at the crop surface [MJ m^{-2} day^{-1}], G stands for soil heat flux density (MJ m^{-2} day^{-1}), T_{mean} stands for the mean daily air temperature at 2 m height (°C); μ_2 is wind speed at 2 m height [m s^{-1}]; e_s and e_a are saturation vapour pressure (kPa) and actual vapour pressure (kPa), respectively, e_s - e_a, the saturation vapour pressure deficit (kPa); Δ, the slope of vapour pressure curve (kPa °C^{-1}) and γ is psychrometric constant (kPa °C^{-1}). The ET_0 was multiplied with crop coefficient (K_c) to calculate estimated crop evapotranspiration (ET_c; mm day^{-1}).

$$ET_c = K_c \times ET_0$$

The K_c is defined as the ratio of crop ET rate to the reference ET rate and K_c values for wheat were taken from FAO Manual 56 (Allen *et al*., 1998).

Actual evapotranspiration (ET_a) was calculated by the following relation;

$$ET_a = K_s\, ET_c$$

Where K_s is the stress factor and ET_c is the crop potential evapotranspiration under standards or no stress conditions. Stress factor (K_s) or evapotranspiration reduction factor ($ET_{red.}$) was calculated by relationship between the relative evapotranspiration reduction [1-(ET_a/ET_m)] and the relative yield reduction [1-(Y_a /Y_m)] using the method given by Doorenbos and Kassam (1979).

The measured amount of irrigation water was applied with the help of cutthroat flumes. The time required to irrigate the field plots to desired irrigation depth was calculated as follows:

$$Qt = Ad$$

Where Q is discharge (m^3 min^{-1}), t is time (min), A is area of plot (m^2) and d is the depth of irrigation (m) which was either $0.7ET_c$, $1.0ET_c$ or $1.3ET_c$.

Measurements and Calculations

Gas exchange attributes: A portable infra-red gas analyzer (IRGA) (model LCi-SD; ADC Bioscientific Ltd., England) was used to measure the photosynthetic rate (A) on a sunny day in the morning between (9:00–11:00 am) at photosynthetic photon flux density of 1200–1400 μ*M* m^{-2} s^{-1} (Ben-Asher *et al*., 2006). Starting from one month after sowing, five measurements were taken at 7-day interval. Measurements were performed in triplicate from each treatment plot on fully expanded youngest leaves of selected plants.

Harvesting and plant analysis: Plant height was recorded in 10 replicates when the crop attained its maximum height. At maturity, after leaving the border rows of 1 m from all the four sides of the plot, net plot (63 m^2) was harvested manually. Crop was threshed to record straw and grain yields and 1000-grain weight. Representative plant samples were collected, oven dried at 70^0C for 72 h and ground in stainless steel mill. Ground plant material was digested in H_2SO_4-H_2O_2 mixture and N was determined using Kjeldahl distillation apparatus.

Water use efficiency: Water use efficiency was calculated in response to evapotranspiration of a specific crop. Crop water use efficiency was calculated as (Howell *et al*., 1990):

$$WUE_{ET} = GY/ET_a$$

Where WUE_{ET} is the water use efficiency based on evapotranspiration (kg ha^{-1} mm^{-1}), GY is the grain yield (kg ha^{-1}) and ET_a (mm) is actual evapotranspiration.

Estimation of crop N recovery: The dired plant samples were digested using wet oxidation method described by Moore and Chapman (1986). Total N concentration was determined using the Kjeldahl distillation and titration method (Bremner and Mulvaney, 1982). Using the grain or straw dry matter yield and concentrations of N, uptake of the N by grain or straw was calculated. These data were utilized to compute crop N recovery as follows (Motavalli *et al*., 1989):

$$\text{Crop N recovery (\%)} = [(N_f - N_c)/F] \times 100$$

Where N_f = N uptake from fertilized plot (kg ha^{-1}), Nc = N uptake from control (kg ha^{-1}) and F = total amount of N applied (kg ha^{-1}).

Soil sampling and analyses: Before sowing and after harvesting of crop, soil samples were collected randomly from each treatment plot. Composite soil samples were characterized for various physical (bulk density, percent porosity, saturated hydraulic conductivity, infiltration rate, penetration resistance and particle size distribution) and chemical properties (total organic carbon, electrical conductivity, pH and total N, available P and available K content). Soil organic carbon was measured by potassium

Table 1: Monthly average weather data druing the study period

Month	Sunshine (h/day)	Reference ET (mm)	Max. temp (ºC)	Min. temp (ºC)	Relative humidity (%)	Rainfall (mm)
December	7	1.7	20.9	4.2	59.1	0
January	7	1.4	17.3	3.2	69.6	3.8
February	7	2.4	18.4	4.6	62.1	8.0
March	8	2.9	25.9	11.7	58.2	1.5
April	9	3.8	32.7	18.0	59.1	7.8
May	10	6.5	38.9	23.3	43.3	0
Total	----	----	----	----	----	21.1

Table 2: Physico-chemical properties of the profile of experiment soil

Property	Depth (cm)	Value
pH_s	-------	7.56
EC_e (dS m^{-1})	-------	1.45
Clay (%)	-------	30
Silt (%)	-------	28
Sand (%)	-------	42
Texture	-------	sandy clay loam
Bulk density (Mg m^{-3})	0-15	1.42
	15-30	1.38
Penetration resistance (kPa)	-------	1011
Total porosity (%)	0-15	46.0
	15-30	48.0
Infiltration rate (mm h^{-1})	-------	25.49
Saturated hydraulic conductivity (Kf_s) (mm h^{-1})	-------	53.2
Available P (Olsen) (mg kg^{-1})	0-15	7.5
	15-30	6.2
Available K (mg kg^{-1})	0-15	111
	15-30	104
Total N (mg kg^{-1})	0-15	0.54
	15-30	0.40
Organic carbon (g kg^{-1})	0-10	4.75
	10-20	2.66
	20-30	1.46
NO_3-N (mg kg^{-1})	0-30	6.3
	30-60	8.1
	60-90	8.7
	90-120	5.2

dichromate method suggested by Ryan *et al*. (2001). Saturated soil paste was prepared and characterized for pH_s and EC_e following the methods described by Richards (1954). For total N in soil method of Bremner and Mulvaney (1982) was adopted. Availabe P was determined by using spectrophotometer (Olsen and Sommers, 1982) and available K was determined by using flame photometer following the method given by (Richards, 1954). Soil texture was determined following the Bouyoucos hydrometer method given by Moodie *et al*. (1959). Soil bulk density was determined by the core method as described by Grossman and Reinsch (2002). Infiltration rate was measured with the help of double ring infiltrometer (Klute, 1986). Soil saturated hydraulic conductivity was measured by Guelph Permeameter (Model 2800 KI), taking three steady-state readings. Soil strength was measured with Eijkelkamp cone penetrometer.

Monitoring of NO_3-N status in soil: The regular monitoring for NO_3-N status of soil was carried out approximately after every month during the growing season for wheat. The soil for NO_3-N analyses was collected at four depths (0–30, 30–60, 60–90 and 90–120 cm) and stored in an ice box till shifting to lab. The well prepared (dried, ground and sieved) soil samples were stored in refrigerator at 0ºC until analysis was done. Soil NO_3-N was extracted with 0.02 *N* copper sulfate, and measured spectrophotometrically (UV-2550, Shimadzu, Japan) using chromotropic acid as color developing reagent (Sims and Jackson, 1971).

Table 3: Rate and time of N fertilizer application to wheat

Rate of N applied (kg ha^{-1})	Time of N application
0	No nitrogen
110	50% at sowing + 50% at tillering*
	50% at sowing + 25% at tillering + 25% at spike initiation
160	50% at sowing + 50% at tillering
	50% at sowing + 25% at tillering + 25% at spike initiation
220	50% at sowing + 50% at tillering
	50% at sowing + 25% at tillering + 25% at spike initiation

***=** maximum tillering. Recommended rate of N for wheat was 110 kg ha^{-1} during the growing seasons of wheat crop in this region

Residual NO_3-N (kg N ha^{-1}) in soil was calculated according to the modified equation by Xue and Hao (2011):

$$\text{Total residual } NO_3\text{–}N = T_i \times BD_i \times [NO_3]_i \times 0.1$$

Where T_i is the thickness of soil layer in cm; BD_i is the bulk density in g cm^{-3}; $[NO_3]_i$ is the soil NO_3–N concentration in mg kg^{-1}, 0.1 is the conversion factor.

Statistical Analysis

The data collected were statistically analysed using Analysis of Variance (ANOVA) technique following randomized complete block design with split plot arrangement (Gomez and Gomez, 1984). Significance was determined for all the analyses at 0.05 probability level. Contrast analysis was done for pairwise comparison among treatments. The software package R was used to analyse the data as well as prepare the graphics.

Resutls

Agronomic Yield and Photosynthetic Rate

The plant height, photosynthetic rate, 1000 grain weight and grain yield of wheat as affected by different treatment combinations (Table 4) showed that the effect of irrigation,

Table 4: Effect of irrigation and nitrogen treatments on crop growth and yield parameters of wheat

Nitrogen	Plant height (cm)				Photosynthetic rate (µmol $m^{-2}s^{-1}$)				1000-grain weight (g)				Grain yield (t ha^{-1})			
	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean
N_0	72.6	82.5	82.9	79.3	8.28	9.45	9.96	9.23	30.9	35.1	35.4	33.8	2.17	2.48	2.36	2.34
N_1S_2	88.7	94.7	95.4	92.2	10.91	13.27	13.06	12.41	38.7	41.1	42.7	40.8	3.12	3.64	3.66	3.47
N_1S_3	88.9	96.1	95.9	93.7	11.68	16.00	16.73	14.81	39.8	42.7	44.3	42.8	3.24	3.85	3.88	3.66
N_2S_2	94.4	98.1	97.8	96.8	12.03	18.58	17.62	16.07	41.5	44.1	45.2	43.6	3.40	4.11	4.25	3.92
N_2S_3	94.9	97.7	99.6	97.4	11.69	19.36	18.31	16.45	42.5	45.3	45.6	44.5	3.51	4.46	4.47	4.15
N_3S_2	94.0	99.0	99.3	97.4	11.16	16.43	15.97	14.52	42.1	44.2	44.1	43.4	3.36	4.01	4.23	3.87
N_3S_3	95.1	99.0	98.7	97.6	11.42	16.32	16.22	14.65	42.2	44.3	44.1	43.6	3.24	4.04	4.23	3.84
Mean	89.8	95.3	95.7		11.02	15.63	15.41		39.7	42.4	43.1		3.15	3.80	3.87	

N_0, N_1, N_2 and N_3 stand for 0, 110, 160 and 210 kg N ha^{-1}. S_2 and S_3 stand for N applied in two splits (50 % +50%) at sowing and maximum tillering and three splits (50 % + 25% + 25%) at sowing, maximum tillering and spike initation of wheat crop

Table 5: Analysis of variance and contrasts for plant height, photosynthetic rate, 1000 grain weight, straw and grain yield of wheat in response to irrigation and nitrogen treatments

SOV	Df	Plant height	Photosynthetic rate	1000 grain weight	Grain yield
		Mean squares	Mean squares	Mean squares	Mean squares
Block	2	4.48	0.299	1.899	9384.6
Irrigation (I)	2	225.63**	141.77**	66.53**	3329011.7***
Error	4	7.43	0.56	0.078	2764.2
Nitrogen (N)	6	388.91**	55.46**	120.85***	3221242.3***
I×N	12	6.19*	4.42**	1.09	68905.4***
Contrasts					
Among treatments at					
I=0.7	6	93.35***	3.56***	23.49***	345413.0***
I=1.0	6	35.13***	10.96***	10.06***	422116.1***
I=1.3	6	31.85***	8.50***	8.44***	623807.4***
Control vs N at					
I=0.7	1	259.49***	3.06**	42.56***	514211.0***
I=1.0	1	5.32NS	32.02***	2.11*	717465.7***
I=1.3	1	5.05NS	21.69***	3.46**	1237137.4***
Among N at					
I=0.7	5	4.72NS	0.63NS	0.46NS	11707.8 NS
I=1.0	5	4.35NS	3.69***	0.21 NS	32317.6 NS
I=1.3	5	3.95NS	3.27***	0.69 NS	55649.7 NS
2 splits vs 3 splits at					
I=0.7	1	4.14NS	4.13NS	1.15 NS	10562.8 NS
I=1.0	1	2.97NS	1.55NS	1.27 NS	77326.7 NS
I=1.3	1	1.99NS	3.20**	0.46 NS	44790.8 NS
Among 3 splits at					
I=0.7	2	22.05**	0.44NS	0.11 NS	20508.2 NS
I=1.0	2	5.54NS	3.66**	0.02 NS	1692.9 NS
I=1.3	2	3.74NS	2.49**	0.41 NS	3570.1 NS
Among 3 splits at					
I=0.7	2	0.07NS	0.32NS	0.15 NS	2041.3 NS
I=1.0	2	4.39NS	3.77**	0.05 NS	18313.8 NS
I=1.3	2	2.69NS	1.59*	0.67 NS	29993.4 NS
Error	36	2.882	0.48	0.41	14217.9

NS = Non-significant ($p \geq 0.05$); * = Significant ($p<0.05$); ** = Highly Significant ($p<0.01$); *** Very highly significant ($p<0.001$). Treatment = N application at four rates either in 2 or 3 splits making 7 treatments. I stands for irrigation

N and irrigation by N interaction on plant height was statistically significant (Table 5). The data regarding plant height averaged over N treatments reveal that the full ($I_{1.0}$) and higher irrigation ($I_{1.3}$) resulted in significantly taller plants than deficit irrigation ($I_{0.7}$), and height of plants was similar at $I_{1.0}$ and $I_{1.3}$ irrigation levels. Plant height increased with increasing rate of N. The tallest plants were recorded in $I_{1.3} \times N_2S_3$ treatment combination. Plant height of wheat showed statistically non-significant differences between and among two or three splits at all the levels of N and irrigation.

Regarding photosynthetic rate (Table 5) data showed that individual as well as interactive effects of irrigation and N were statistically significant ($p<0.001$). The photosynthetic rate varied from 8.28 µmol m^{-2} s^{-1} in $N_0I_{0.7}$ to 19.36 µmol m^{-2} s^{-1} in $N_2S_3I_{1.0}$. It increased up to 160 kg N ha^{-1} and slightly decreased above this N rate. Contrast analysis (Table 5) showed that photosynthetic rate for the control plots (0 kg N ha^{-1}) was less than N treated plots and there were significant differences among all the N treatd plants. Further, there were non-significant differences in photosynthetic rate in response to N application in two and three splits at deficit and full irrigation regimes, however, split applications displayed significant results at high irrigation level ($I_{1.3}$). Averaged across irrigation levels, the

Table 6: Effect of irrigation and nitrogen on ET (mm), D (mm), WUE (mm kg ha^{-1}) and CNR (%) of wheat

Treatments	Seasonal ET				Drainage losses				Crop water use efficiency (WUE)				Crop N recovery (CNR)			
	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean	$I_{0.7}$	$I_{1.0}$	$I_{1.3}$	Mean
N_0	267.0	315.1	362.4	314.9	7.72	16.58	20.58	14.96	8.12	7.87	6.51	7.50	-	-	-	
N_1S_2	282.5	332.4	373.6	329.5	5.23	13.22	17.30	11.92	11.03	10.95	9.79	10.59	35	47	50	44
N_1S_3	294.0	343.9	385.4	341.1	3.86	11.10	14.37	9.78	11.03	11.20	10.08	10.77	45	56	58	53
N_2S_2	311.1	354.6	401.1	355.6	2.82	8.92	12.09	9.94	10.92	11.60	10.60	11.04	36	49	51	46
N_2S_3	323.6	367.3	411.6	367.5	1.92	7.54	9.54	6.33	10.86	12.13	10.87	11.29	39	58	58	51
N_3S_2	312.0	360.3	403.8	358.7	1.96	7.23	9.39	6.19	11.19	11.87	10.48	11.18	27	36	39	34
N_3S_3	311.9	361.2	404.4	359.1	2.08	7.49	9.13	6.23	11.24	11.56	10.54	11.12	25	36	38	33
Mean	300.3	347.8	391.7		3.66	10.30	13.20		10.63	11.03	9.84		35	47	49	

N_0, N_1, N_2 and N_3 stand for 0, 110, 160 and 210 kg N ha^{-1}. S_2 and S_3 stand for N applied in two splits (50 % +50%) at sowing and maximum tillering and three (50 % + 25% + 25%) splits at sowing, maximum tillering and spike inititaiton stages of wheat crop, respectively

Table 7: Analysis of variance and contrasts for seasonal ET, Drainage losses, WUE and CNR

SOV	df	Seasonal ET Mean squares	Drainage Mean squares	WUE_{ET} Mean squares	CNR Mean squares
Block	2	3.54	0.50	0.029	36.70
Irrigation (I)	2	43929.64***	502.86***	7.657***	951.19***
Error-1	4	0.41	0.07	0.112	16.69
Nitrogen (N)	6	3229.17***	102.62***	16.252***	3552.96***
I×N	12	9.09***	4.21***	0.298 ^{NS}	34.98***
Contrasts					
Among treatments at					
I=0.7	6	182.70***	3.86***	2.891***	714.95***
I=1.0	6	138.90***	9.25***	2.729***	631.69***
I=1.3	6	103.23***	16.47***	2.931***	692.01***
Control vs N at					
I=0.7	1	258.53***	4.42***	6.455***	774.54***
I=1.0	1	59.10***	17.39***	4.998***	862.19***
I=1.3	1	9.66 ^{NS}	32.28***	6.145***	1009.36***
Among N at					
I=0.7	5	40.28***	0.67**	0.036 ^{NS}	47.41***
I=1.0	5	38.11***	2.73***	0.215 ^{NS}	21.40***
I=1.3	5	33.12***	7.49***	0.206 ^{NS}	20.38***
2 splits vs 3 splits at					
I=0.7	1	40.89***	0.40 ^{NS}	0.084 ^{NS}	105.02***
I=1.0	1	24.04***	0.84*	0.000 ^{NS}	89.37***
I=1.3	1	15.72**	4.13***	0.006 ^{NS}	57.36***
Among 3 splits at					
I=0.7	2	0.58 ^{NS}	0.04 ^{NS}	0.011 ^{NS}	26.19***
I=1.0	2	6.54 ^{NS}	1.46***	0.092 ^{NS}	12.25 ^{NS}
I=1.3	2	0.08 ^{NS}	4.15***	0.114 ^{NS}	15.98 ^{NS}
Among 3 splits at					
I=0.7	2	1.11 ^{NS}	0.05 ^{NS}	0.154 ^{NS}	8.18 ^{NS}
I=1.0	2	5.03 ^{NS}	0.73 ^{NS}	0.636 ^{NS}	6.99 ^{NS}
I=1.3	2	2.17^{NS}	2.50***	0.504^{NS}	1.95^{NS}
Error	36	1.54	0.15	0.118	2.981

NS = Non-significant ($p \geq 0.05$); * = Significant ($p<0.05$); ** = Highly Significant ($p<0.01$); *** Very highly significant ($p<0.001$). Treatment = N application at four rates either in 2 or 3 splits making 7 treatments. I stands for irrigation

photosynthetic rate remained higher with N application in three splits compared to N application in two splits and these differences were much higher at lower level of N (110 kg N ha^{-1}) than at higher levels of N (160 kg N ha^{-1} and 210 kg N ha^{-1}). The mean photosynthetic rate was only 11.02 in $I_{0.7}$, which increased to 15.63 µmol m^{-2} s^{-1} with $I_{1.0}$ (higher by 42% than I0$_{.7}$) and to 15.41 µmol m^{-2} s^{-1} with $I_{1.3}$ (higher by 40% than $I_{0.7}$).

Both individual as well as interactive effects of irrigation and N was significant (p= 0.012) on 1000-grain weight (TGW). The TGW ranged from 30.9 g in $N_0I_{0.7}$ to 45.6 g in $N_2S_3I_{1.3.}$ The grain weight increased up to 160 kg N ha^{-1} and above these levels, a slight decrease in grain weight was observed. Contrast analysis (Table 5) showed that crop treated with N irrespective of rate and timing produced significantly heavier grains than control (0 kg N ha^{-1}) at all levels of irrigation. The TGW was higher with N application in three splits compared to N application in two splits, however, difference between three and two splits was much higher at lower rate of N (110 kg N ha^{-1}) than at higher rates (160 kg N ha^{-1} and 210 kg N ha^{-1}). Averaged across N treatments for the three irrigation levels, TGW was only 39.7 g with $I_{0.7}$, which increased to 42.4 g with $I_{1.0}$, 7% higher than $I_{0.7}$ and then to 43.1 g with $I_{1.3,}$ higher

Table 8: Analysis of variance for NO_3-N kg ha^{-1} in soil profile at various growth stages of wheat

SOV	Df	Mean squares
Block	2	0.26
Irrigation (I)	2	4556.21***
Error-1	4	3.47
Nitrogen (N)	6	19081.74***
I×N	12	78.03***
Error	36	1.37
Depth (D)	3	9790.92***
Error	6	2.37
I×D	6	149.73***
Error	12	1.05
N×D	18	211.84***
Error	36	1.38
I×N×D	36	5.72***
Error	72	2.15
Sampling date (SD)	3	1114.39***
Error	6	1.19
I×SD	6	40.81***
Error	12	0.73
N×SD	18	191.83***
Error	36	1.02
I×N×SD	36	8.14***
Error	72	1.83
D×SD	9	229.99***
Error	18	0.983
I×D×SD	18	16.89***
Error	36	1.27
N×D×SD	54	16.00***
Error	108	1.07
I×N×D×SD	108	5.38***
Error	216	1.39

NS = Non-significant ($p \geq 0.05$); * = Significant ($p<0.05$);
** = Highly Significant ($p<0.01$); ***Very highly significant ($p<0.001$)

by 8% than $I_{0.7}$.

There was significant effect of irrigation, N and their interaction on grain yield of wheat. It varied from 2.17 in $N_0I_{0.7}$ to 4.47 in $N_2S_3I_{1.3}$ (Table 4). Contrast analysis (Table 5) showed that grain yield was significantly ($p<0.001$) affected by treatments at all levels of irrigations. The difference between the yield from N treated and those of control plots was significant only at deficit ($I_{0.7}$) and full irrigation ($I_{1.0}$). However, statistically similar yield was recorded for treated and non-treated plots at higher irrigation ($I_{1.7}$). Greater differences in GY occured across N treatments as compared with irrigation levels. It is noteworthy that N rate of 160 was sufficient to maximize the grain yield at all levels of irrigation. In the current study, regardless of timing of N application, yield did not improve (even decreased slightly) above the 160 kg N ha^{-1} rate. Averaged over irrigation levels, the grain yield was higher with N application in three splits compared to two splits at all the rates of N except 210 kg N ha^{-1}, where almost similar or even less yield was obtained with three splits of N.

Crop N Recovery

Recovery of N ranged from 25% in $N_3S_3I_{0.7}$ to 58% in $N_2S_3I_{1.0}$ and was at par with $N_2S_3I_{1.0}$. Contrast analysis for crop N recovery showed significant variations among the N

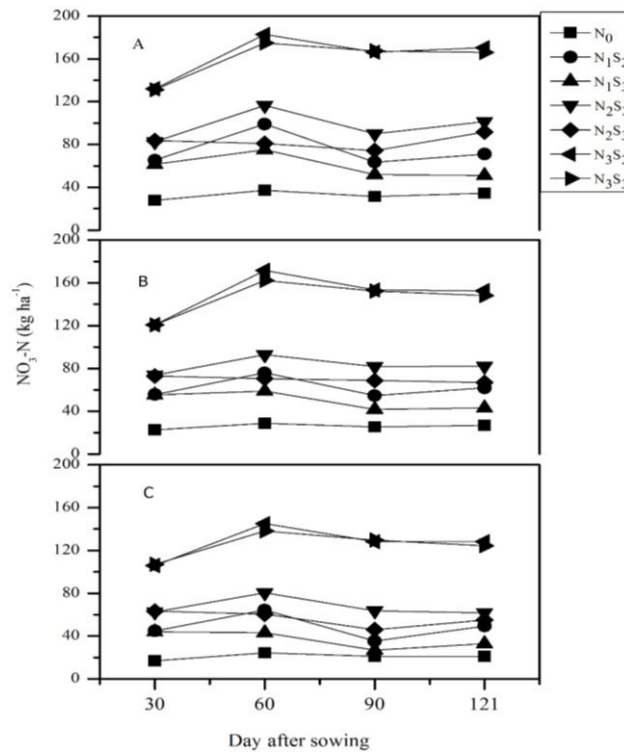

Fig. 1: Effect of irrigation and N on NO_3-N in soil measured at various times during growth season of wheat crop. A, B and C stand for deficit, full and excessive irrigations, respectively

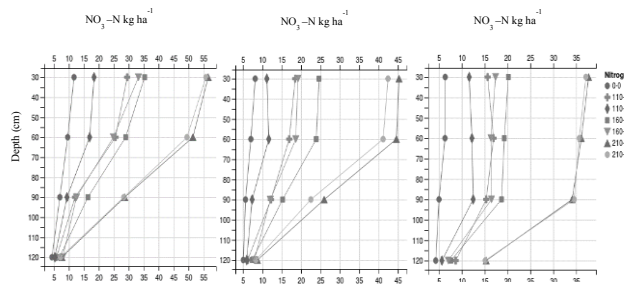

Fig. 2: Soil NO_3 profile in different water and N regimes, averaged over all sampling dates

treatments at all levels of irrigation. Significant differences were observed between two splits and three splits of N at various levels of irrigation. Application of N at 110 and 160 kg ha^{-1} in three splits resulted in 20% and 11% more N recovery compared to the same rates of N applied in two splits. Therefore, if N is applied in three splits, it would be possible to save 50 kg N ha^{-1} without compromising yield and crop N utilization. In order to further clarify the effect of irrigation on N recovery, data were averaged across N treatments. The N recovery was only 35% in deficit irrigation ($I_{0.7}$), which increased to 47% in full irrigation ($I_{1.0}$) (higher by 34% than $I_{0.7}$) and then to 49% in higher irrigation ($I_{1.3}$).

Crop Water Use and Drainage

A significant effect of irrigation, N and their interaction on ET and D of wheat crop was found (Table 7). Plots treated with N irrespective of rate and timing registered significantly greater ET than of plots without N (0 kg N ha^{-1}) at all irrigation levels. When N rate surpassed a certain threshold (160 kg N ha^{-1}), the total ET greatly decreased, indicating that ET could not increase further if too much N was applied. The seasonal ET ranged from 267.0 mm ($N_0I_{0.7}$) to 411.6 mm ($N_2S_3I_{1.3}$). The seasonal ET values were higher in three splits of N compared to the two splits of N. The total crop ET increased linearly with an increase in irrigation levels. The mean ET was 300.3 mm in $I_{0.7}$, which increased to 347.8 mm with $I_{1.0}$ (higher by 16% than $I_{0.7}$) and then to 391.7 mm with $I_{1.3}$ (higher by 30 and 21% than $I_{0.7}$ and $1_{1.0}$, respectively).

With an increase in level of fertilization, the D values decreased since increase in level of fertilization increased the crop water use (ET). At similar level, N application in three splits resulted in considerably lower values of D as compared to two splits of N. For instance, at same level of N (110 kg N ha^{-1}), the D loss was 11.92 mm with two splits of N, which decreased to 9.78 mm with three splits of N (lower by 22 % than two splits). The difference between two and three splits of N was observed only up to N application of 160 kg ha^{-1}. Irrigation had significant impact on drainage losses of water and D increased linearly with increasing irrigation level. The data revealed that D loss was only 3.5% in deficit irrigation ($I_{0.7}$), which increased to 10.3 % in full irrigation ($I_{1.0}$) and then to 49% in higher irrigation ($I_{1.3}$).

Water Use Efficiency

Results (Table 7) indicated statistically significant effect of irrigation, N and interactive effect of irrigation and N on WUE_{ET} of wheat crop. Contrast analysis (Table 7) showed that ET based WUE was significantly ($p<0.001$) affected by N treatments at all levels of irrigations. Likewise, plots treated with N irrespective of rate and timing registered significantly greater WUE_{ET} than the WUE_{ET} without N (0 kg N ha^{-1}) at all irrigations. Statistically non-significant differences were observed between two splits (average of N_1S_2, N_2S_2 and N_3S_2: whole of N in two equal splits, i.e. 50% + 50%) and three splits (average of N_1S_3, N_2S_3 and N_3S_3: whole of N in three unequal splits, i.e. 50% + 25% + 25%) at various levels of irrigation. The results further showed that both with the increase in water as well as N supply, the WUE_{ET} tended to increase, however, the increase ceased when the water and N supply reached a certain level (Table 6). It is noteworthy that full irrigation ($I_{1.0}$) and N rate of 160 kg N ha^{-1} was sufficient to maximize the WUE_{ET}. In the present study, both the deficit as well as the excessive irrigation registered lesser WUE_{ET} than the full irrigation.

Temporal Variation of NO_3-N

The temporal vriation of soil NO_3^- from initiation to completion of the experiment has been shown in Fig. 1. The data analysis of NO_3^- over growth stages of wheat (Table 8) show significant effects of irrigation, nitrogen, soil depth, sampling date and all possible interactions.

Regardless of irrigation levels, three splits of N resulted in lower levels of residual NO_3^- in soil as compared to two splits of N. Further, the effect of split application on NO_3-N in soil could not be found until 60 days after sowing (DAS). This could be due to the fact that until 60 DAS, all the treatment plots (irrespective of two or three splits) received equal amount of N (50% of the total N applied). Irrespective of irrigation and split application: soil NO_3^- was found to be greater under the highest levels of N (N_3: 210) followed by N_2 (160 kg N ha^{-1}), N_1 (110 kg N ha^{-1}) and N_0 (control) throughout the study period. Irrigation showed significant effect on NO_3-N content in soil (120 cm) throughout the growth period of wheat. There was much higher build up of residual NO_3-N in 1.2 m soil profile with $I_{0.7}$, as compared to $I_{1.0}$ and $I_{1.3}$. For example, at 60 DAS, residual NO_3^- was only 63.51 kg ha^{-1} with $I_{1.3}$, which increased to 74.59 kg ha^{-1} with $I_{1.0}$ (higher by 17% than $I_{1.0}$) and then to 83.37 kg ha^{-1} with $I_{0.7}$ (higher by 11% & 31 % than $I_{1.0}$ and $I_{1.3}$). Similar effects of irrigation levels on accumulation of NO_3^- in soil profile were found for all the sampling dates during the growing season of wheat.

Distribution of NO_3-N in Soil Profile

Concentration of NO_3–N in the subsurface layers was considerably lesser than surface layers in all the treatments (Fig. 2). Irrespective of splits and irrigation levels, the NO_3–N concentration increased with increasing rate of N and treatment N_3 (210 kg N ha^{-1}) had a significant higher NO_3–N concentration than other N treatments in the 0–120 cm depth soil profile. Such accumulation of NO_3–N in the soil profile may result in excessive N leaching into deeper soil layers during monsoon or high irrigation levels. The data also depicted that the three splits of N resulted in considerably lower soil NO_3–N than the two splits of N however; the considerable differences were noted up to 0-90 cm soil depth. Below 0–90 cm soil depth, the differences between two and three splits decreased. Moreover, the differences between two and three splits were true for only lower levels of N (N1 and N2). At highest levels of N application (210 kg ha^{-1}), there was no considerable difference between the soil NO_3-N in two and three splits at all levels of irrigation.

The soil NO_3–N contents were substantially higher for deficit irrigation than full and higher irrigation levels at all soil depths. Higher irrigation levels lead to more NO_3-N leaching from root zone, resulting lower soil NO_3-N concentrations in surface layers of soil at harvest. The similar fashion for depth distribution of NO_3-N was found in

all the the treatments. Regarding depth distribution of NO_3–N in deficit and full irrigation, there was no differences in concentration upto 0–60 cm soil depth, however, below this depth concentration decreased sharply.

Discussion

The results from current experiment showed that full and higher irrigation along with 160 kg N ha^{-1} increased the height of wheat plants compared to other treatments. One plausible explanation for these results is that high and optimum irrigation levels resulted in better availability of applied N, leading to more cell division and enlargement and consequently the taller plants (Kirda *et al*., 2005). Gheysari *et al*. (2009) reported increase in plant height with the increasing levels of irrigation and N. Our results showed non-sginficant impact of split applications on plant height. However, these results are contrary to the findings of Niaz *et al*. (2014) who reported increase in plant height with increasing number of nitrogen splits. These contradictory results might be due to variation in rate and timing of N application, climatic conditions and the species and cultivars employed for experimentation.

An increase in photosynthetic rate with N could be attributed to beneficial impact of N on photosynthetic apparatus. Sugiharto *et al*. (1990) reported a significant positive correlation between leaf N concentration and their photosynthetic capacity suggesting critical role of N in synthesis of components of the photosynthetic apparatus. Shangguan *et al*. (2000) reported an increase in mean photosynthetic rate in wheat and maize with increasing rate of N application. Further, the current study added important evidence in the interactive effects of N addition and soil water on the photosynthetic rate of wheat. Cabrera-Bosquet *et al*. (2007) also reported significant positive interaction of N and soil water on the photosynthetic rate in durum wheat.

In our experiment, grain weight increased with increasing rate of N, but the positive impact of N on grain weight was accelerated by high water availability and decreased by water shortage. This could be attributed to increased availability and uptake of N under optimum water availability (Di Tomaso, 1995). Fertilizer-N improved grains weight but excessive use was not beneficial as reported by Brown and Petrie (2006) who showed that plants supplied with excessive N produced more vegetative growth and acquired less kernel weight. Considerable increase in grain weight with the application of N in three splits may be attributed to enhanced availability of N for a longer period in soil due to less leaching and volatilization losses of N. These factors enable the plants to synthesize more photosynthates at later stage, which in turn, were translocated to produce large sized gains (Lopez-Bellido *et al*., 2006).

Results reveled that grain yield increased with N application and the amount of water supplied influenced the response to N, i.e. at the same level of N application, grain yield was more for full and higher irrigation than grain yield at deficit irrigation. Al-Kaisi *et al*. (2005) reported that the optimum N rate for maximum crop yield was the same under different irrigation conditions. The same optimum N rate for maximum yield under different irrigation conditions could be due to the decrease in NUE as soil water content decreased. In the current experiment, regardless of crop season and timing, yield did not improve (even decreased slightly) above N application of 160 kg ha^{-1}. The decrease in grain yield with higher rates particularly at sowing could be attributed to increaseed straw yield, and this might have depleted moisture contents and contributed to yield decrease (Kalra *et al*., 2007).

Split application of N is more beneficial in terms of grain yield at lower level of N than splitting the higher levels of N fertilization (Binder *et al*., 2000). Nitrogen availability throughout the growing period of a crop is necessary to attain good crop productivity. Delayed or early application of N does not allow sufficient time for plant activities related to physiological, phenological, agronomic and N uptake traits thus leading to lower crop production. Moreover, application of N during earlier growth stages can result in range of possible losses due to its greater immobilization, leaching, and clay fixation. The increase in grain yield of wheat with the three splits of N fertilizer may also be related to improved photosynthesis and grain weight (Table 4) resulting from enhanced availability for uptake (Table 6) throughout the growing season (Hooper *et al*., 2015) and also less losses of N from leaching.

The utilization of applied N by wheat was evaluated in terms of CNR. The N recovery values recorded in the present investigation is within the range reported by Fischer *et al*. (1993) and Moser (2004). A reduction in CNR with increase in fertilizer dose has been reported by other workers. Lenka *et al.* (2013) reported reduction of apparent fertilizer N recovery in wheat and maize crops with increase in fertilizer dose. The reduction in CNR with increasing dose may be attributed to more leaching and volatilization losses of N with the application of N more than crop needs. The increase in CNR with three splits of N could be readily explained by more uptake and less leaching losses of N (Lopez-Bellido *et al*., 2006).

Application of N irrespective of rate and timing registered significantly greater ET than the ET of control at all irrigation levels. The response of ET to N fertilization was almost similar to the response of grain yield to N fertilization, i.e. when N rate surpassed a certain threshold (160 kg N ha^{-1}), the total ET greatly decreased, indicating that ET could not increase further if too much N was applied. This could be attributed to the fact that N promotes both shoot and root growth and enabling more soil water to be absorbed (Gajri *et al*., 1989). Another explanation could be that N fertilization increases the leaf area index and transpiration rates of wheat. However, N applied at level above than recommended makes soil environments stressful by increasing N concentration in soil solution, thus

preventing roots from absorbing water. The ET values noted in this study are well close to reported by other researchers (Lenka *et al*., 2009; Zhong and Shungguan, 2014). However, these values are higher than reported by Behera and Panda (2009). The greater ET with three splits of N may be related with the much higher shoot biomass and root growth that resulted in higher ET. Similarly greater availability of soil water with full and higher irrigations than with deficit irrigation increased plant growth and thereby resulted in higher amount of evapotranspiration. These observations are in agreement with the findings of Ram *et al*. (2013) who reported that ET of wheat increased from 289 mm with two irrigations to 512 mm with five irrigations in Indian Punjab.

The lesser drainage losses in three splits of N as compared with two splits of N may be due to the fact that application of N in three splits resulted in better crop growth and hence better utilization of applied water with former. Irrigation had significant impact on drainage losses and D increased linearly with increasing irrigation level. Similarly, an increase in D losses with increase in irrigation water has also been reported in wheat and maize crops (Behera and Panda, 2009).

Our results showed increase in WUE_{ET} with N fertilization. Similar to our reults, the increase in WUE_{ET} with N fertilization has been reported by Albrizio *et al*. (2010). The decrease in WUE_{ET} with the highest levels of N (210 kg N ha^{-1}) at all levels of irrigation could be ascribed to the decrease in grain yield with excessive levels of N fertilization. Unexpected reduction in WUE_{ET} with deficit irrigation may be related to grain yield decline with water stress that resulted in lower $WUE_{ET.}$ Oweis *et al*. (2011) reported severe decline in WUE_{ET} under water stress. The authors postulated that at lower irrigation levels, plants remaining under water stress used less water and produced low grain yield with low WUE_{ET} indices. The decline in WUE_{ET} with excessive irrigation compared with deficit and full irrigation may be due to relatively greater evapotranspiration (ET) than the corresponding increase in grain yield. Zwart and Bastiaansen (2004) reported a decrease in WUE_{ET} with water supply at levels more than the crop evapotranspiration demands.

The residual soil NO_3^- under highest level of N (irrespective of splits) also led to a lower crop N recovery. A discernable build up of residual NO_3–N in soil in response to overdose of N application has been reported in other parts of the world (Andraski *et al*., 2000; Wang *et al*., 2010; Gholamhoseini *et al*., 2013b). The build up of residual soil nitrate to the extent of 213 kg N ha^{-1} in 120 cm soil depth was reported from two years wheat-maize cropping system in India (Lenka *et al*., 2013). Likewise, Cui *et al*. (2010) reported build up of residual NO_3-N in soil profile in response to continuous application of excessive N in North China Plain. Similarly, lower levels of residual NO_3^- were reported in high irrigation treatment than in low irrigation treatment at 120 cm (Lenka *et al*., 2013) and 200 cm (Wang *et al*., 2010) soil profile. In contrast, higher NO_3^- accumulation was observed under irrigation (135 kg N ha^{-1} yr^{-1}) as compared to dry land conditions (82.5 kg N ha^{-1} yr^{-1} in the 0–400 cm soil profile (Fan *et al*., 2010). These discrepancies may be related to the differences in sampling depth in these experiments. In our experiment, sampling depth was restricted to 120 cm soil depth and thus it may be possible that irrigation helped leaching of some NO_3^- below the 120 cm soil depth.

This situation warrants the judicious use of N fertilizer both under deficit and excessive irrigation conditions. Excessive application of N in water scarce conditions may result in lower crop yield and N recovery and thus leading to soil NO_3^- build up. However, excessive application of N in over irrigation situations, may lead to leaching of N with water passing the soil profile and polluting the upper groundwater.

Conclusion

Our results showed the potential of optimizing of N the rate, application timings and irrigation in an effort to reduce N losses without decreasing yield, or in a best scenario with an increase in yield. The promising results regarding the effects of three splits of N along with water application according to crop water requirement (ET_c) necessitates the evaluation of these factors under various agro-ecological and soil conditions in Pakistan. The potential of NO_3 leaching in response to high levles of N and irrigation water under different cropping systems has not been evaluated. Long term field studies under different agricultural practices to monitor the build up and leaching of NO_3 should be conducted.

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