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Original article

Deficit irrigation and nitrogen effects on nitrogen-use efficiency and grain protein of rice

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Abstract – To meet future food demand, world rice production must increase in the next few decades, which is possible only by effective use of soil and water resources and inputs. This research was conducted to investigate the effects of nitrogen, N, application and deficit irrigation treatments including a sprinkler, intermittent flooding and continuous flood irrigation, and their interaction with the N-use efficiency and grain protein of a local lowland rice cultivar. The results indicated that low (sprinkler irrigation) and high (continuous flood irrigation) applied water affected the plant and soil factors in N uptake and decreased N-use efficiency for rice. Therefore, optimum applied water was obtained in intermittent flooding (2-day interval). Reduction in nitrogen uptake at high applied water can be due to the effect of nitrate leaching in the root zone and the reduction in N uptake at low applied water can be due to the inability of the roots to absorb N and translocate it to the plant top. With respect to the relationship between N uptake and grain protein and leaf chlorophyll, these parameters can also be affected by applied water and N application. Appropriate linear models were proposed to show these relationships. At different times of soil nitrogen measurements and N application rates, maximum nitrogen leaching (about 50%) occurred in continuous flooding irrigation.

deficit irrigation / nitrogen-use efficiency / grain protein / rice

1. INTRODUCTION

Rice (Oryza sativa L.) is the world's most important food crop and a major food grain for more than a third of the world's population. More than 70% of the world's rice is produced in intensively cultivated, irrigated lowland systems in Asia [11]. To meet future demand, world rice production must increase in the next few decades, which is possible only if soil and water resources and inputs are used more efficiently. In these systems, nitrogen, N, and water are two of the main factors limiting the realization of yield potentials [12, 5]. As a consequence, large amounts of mineral N fertilizers are used. According to one estimate, 7×10^6 metric tons of N are applied each year to the 74×10^6 ha of irrigated rice in Asia [3]. Apparent recovery (RE) of fertilizer N by rice varies widely from 0 to 100%, while agronomic efficiency (AE) ranges from 0 to 45 kg grain per kg N applied [8]. Vlek and Byrnes [24] stated that "in field experiments, flooded rice generally recovers 20-40% of applied N, whereas upland crops normally recover about 40-60%".

With the present levels of fertilizer N-use efficiency (<40%) in rice, it is estimated that an increase of about 300% in N use will be required to achieve an average yield of 8 t ha⁻¹ by 2025 [3]. Nitrogen fertilization in rice is expensive throughout the world, but fertilizer N recovery is seldom more than 30–40% under normal conditions and 60–65% under optimum condi-

tions [10]. According to Timsina et al. [22], agronomic N-use efficiency (kg grain yield per kg N applied), physiological efficiency (kg grain yield per kg N absorbed), and fertilizer N-recovery efficiency (kg N absorbed per kg N applied, expressed as %) for rice across treatments ranged from 2.8 to 10.8, 5.2 to 27.5, and 33 to 61, respectively, and all were greater for N application at 90 compared with 135 kg N ha⁻¹. All those parameters under irrigated conditions had greater values than rainfed conditions. In West Africa [26], apparent recovery of fertilizer N was highly variable (average: 30–40% of applied N) and physiological nitrogen efficiency was mostly between 40 and 80 kg grain kg⁻¹ plant N. Cassman et al. [4] reported that mean N uptake efficiency from different N fertilizers was only 36%.

Reduction in N use may be possible in some areas without any sacrifice in yields [25]. In general, a given percentage increase in yield will do much more for profitability than a similar percentage reduction in N use, because the ratio of N costs to gross revenue from paddy is typically 8% or less [9]. Reduced use of N fertilizer might also generate off-farm environmental benefits.

The chlorophyll or SPAD meter is a promising tool for analysis of the nitrogen status of a crop. It could help to improve grain yield and N-use efficiency [1, 16]. The SPAD-502 CHLOROPHYLL METER determines the relative amount of chlorophyll in leaves by measuring transmittances at red (650 nm,

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where absorption is high) and near-infrared (940 nm, where absorption is extremely low) wavelength regions [13]. Researchers have widely used SPAD meter readings to predict chlorophyll concentrations in rice and other crops [17]. Since leaf chlorophyll is highly correlated with N concentration, the SPAD meter has also been used for analysis of the N fertilizer status of rice [7, 21, 23].

The problems in rice productivity are both rice production quantity and quality, i.e., grain protein content. Although applied water and nitrogen fertilizer influence many quality criteria, their effect on the protein content in rice grain may be of the greatest significance. In general, not much data have been reported for the effects of deficit irrigation and nitrogen application rate on nitrogen-use efficiency and grain protein of rice, in the literature. Therefore, this research was conducted to investigate the effects of N application and deficit irrigation treatments including a sprinkler, intermittent flooding and continuous irrigation, and their interactions with the N-use efficiency and grain protein of a local lowland rice cultivar.

2. MATERIALS AND METHODS

2.1. Site description

This research was conducted at the Kooshkak Agricultural Research Station of Shiraz University (Lat. 30° 7' N; Long. 52° 34' E; 1650 m El.) during the two consecutive growing seasons of 2000 and 2001. The experimental site was placed in the irrigated area of the Doroodzan Irrigation District located south of I.R. of Iran. The soil of the experimental site was a Fine, Carbonatic, mesic, Aquic Calcixerepts soil with a pH of 6.9-7.1. The soil contents of sand (%), silt (%) and clay (%), electrical conductivity of saturation extract (dS m⁻¹), pH, N-NO₃ (kg ha⁻¹), $N-NH_4$ (kg ha⁻¹) and P (kg ha⁻¹) were 30, 39, 31, 1.4, 7.1, 12.8, 3.3, and 17.8, respectively, at a depth of 0-30 cm. These values at a depth of 30-60 cm were 25, 32, 43, 1.1, 6.9, 10.7, 4.2 and 10.9, respectively. Maximum temperatures during the growing season (July-October) ranged from 23 to 39 °C in 2000 and from 28 to 40 °C in 2001, while the minimum temperature ranged from 7 to 24 °C in 2000 and from 8 to 27 °C in 2001, respectively. The mean daily maximum and minimum temperatures during the growing season (July-October) were 32.8 and 13.2 in 2000, respectively. These values were 35.4 and 16.2 °C in 2001, respectively. Reference potential evapotranspirations (ET_p) during the growing period for 2000 and 2001 were 578 and 650 mm, respectively. There was no rainfall during the growing season in either year.

2.2. Experimental details

The experiment was conducted using four replications in a split-plot design with the irrigation methods as the main plots and N levels as subplots. The main plots consisted of five irrigation regimes: (1) sprinkler irrigation with applied water equal to ET_p , (2) sprinkler irrigation with applied water equal to 1.5 ET_p , (3) continuous flooding irrigation, (4) intermittent flooding irrigation with a 1-day interval, and (5) intermittent flooding irrigation with a 2-day interval. The one- and two-day intervals are the periods between the consecutive flooding of

the plots with a water height of 5 cm. All the plots were bounded to prevent any water runoff. The 1.0 and 1.5 ET_{p} treatments did not lead to runoff flooding. The percolation rate under surface flooding was determined at $3-4 \text{ mm d}^{-1}$ [18]. The subplots were composed of three levels of 0, 40, and 80 kg N ha⁻¹ for 2000 and 0, 60, and 120 kg N ha⁻¹ for 2001 applied as urea. Subplots were $3 \text{ m} \times 3 \text{ m}$ basins enclosed by 50-cm bunds. The land was prepared on 8 to 10 July in 2000 and 28 to 30 June in 2001. A local cultivar (Champa-Kamphiroozi, Indica, with no nematode infection) of rice seedlings with low tillering ability were transplanted, with 16 and 25 bushes per unit area, m² for 2000 and 2001 on 11 and 1 July in 2000 and 2001, respectively. Before transplanting, 200 kg P ha⁻¹ was applied as ammonium phosphate. The N associated with this fertilizer was not counted in the N treatments, which were based on urea. For the first ten days, all of the treatments were irrigated with continuous flooding to establish the seedlings. The applied water in this period was 142 and 166 mm in the years of 2000 and 2001, respectively.

For sprinkler treatments, the applied water for each irrigation was obtained by the mean of ET_p for the three previous days plus evaporation and the wind drift losses of the sprinklers. Evaporation and wind drift losses were determined by measuring the water collected in 45 cans placed in the experimental plots during the water application period. The difference between the applied water and the water collected in the cans was considered as evaporation and wind drift losses. The mean value of this loss was 28.8% in the growing season. For flooding treatments, the water depth in the plots was maintained at 5 to 10 cm in the irrigation period.

Ten days after transplanting, one-half of N per ha and irrigation treatments were applied. The remaining N was applied in the middle of the growing season. In 2001, to monitor the greenness of the crop in response to N status, a chlorophyll meter (SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Ltd., Japan) was used to take SPAD readings at, before and after the second N application. At the measuring time, the SPAD readings were taken on the five uppermost fully expanded leaves from different plants in each plot. Three chlorophyll meter readings were taken around the midpoint of each leaf blade, 30 mm apart, on one side of the midrib. Therefore, fifteen SPAD readings were averaged to represent the mean SPAD value of each plot. When the SPAD readings were recorded, these leaves from each plot were clipped and pooled to measure N concentration by micro-Kjeldahl digestion and distillation [2] after oven-drying at 70 °C. During the growing season, weeds were controlled by hand-weeding. Soil samples from each plot were taken at a depth of 0-30 and 30-60 cm before the second N application and at the end of the growing season. N-NO3 and N-NH4 of samples were measured by the colorimetric method [14].

A volumetric water meter measured the volume of the delivered water to the plots. The crop was harvested manually on 8 and 13 October in 2000 and 2001, respectively. At the end of the growing season, yield samples were harvested from a 1 m \times 1 m area in the middle of the plots. Samples were air-dried for 5 days before being oven-dried at 70 °C for 48 h. Then, grain and straw N was determined on composite samples from all replications from every treatment combination using the micro-Kjeldahl digestion and distillation procedure. The percent of

	_		Ν	applied (kg	ha ⁻¹)		
Year	Irrigation treatment	32		72		112	
	Sprinker (1*ETp)	1728	efg*	1643	efg	1614	efg
	Sprinkler (1.5*ETp)	1371	g	1488	fg	2258	de
2000	Continuous flooding	3821	а	3318	ab	3707	ab
	Intermittent fl.(1d. Int.)	2141	def	2150	def	3072	bc
	Intermittent fl.(2d. Int.)	1698	efg	2686	cd	3587	ab
			N	applied (kg	ha ⁻¹)		
		32		92		152	
	Sprinker (1*ETp)	3541	f	3353	f	3745	ef
	Sprinkler (1.5*ETp)	3476	f	3726	ef	4293	cde
2001	Continuous flooding	4196	de	4838	bc	5964	а
	Intermittent fl.(1d. Int.)	4262	cde	4885	bc	6061	а
	Intermittent fl.(2d. Int.)	4448	cd	5085	b	5975	а

Table I. Grain yields (kg ha^{-1}) in different irrigation and nitrogen treatments for 2000 and 2001.

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

grain protein was obtained from the grain N multiple by a coefficient of 6.25 [2].

Physiological efficiency (PE, kg grain yield per kg N absorbed), fertilizer N-recovery efficiency (RE, kg N absorbed per kg N applied), and agronomic N-use efficiency (AE, kg grain yield per kg N applied) were calculated for various treatments. In calculating the AE and RE terms, the zero-N treatment is used as reference (internal standard) within the replication (block). PE reflects the efficiency in using the N actually absorbed and approximates the effects of plant factors, RE focuses on N absorption from applied N and explains the effects of soil factors, and AE reflects the efficiency of applied N [15].

2.3. Statistical analysis

All the plant data collected were statistically analyzed as a split-plot design with four replications using analysis of variance to evaluate main and interaction effects. Means between treatments were compared using the Duncan multiple range test at the $P \le 0.05$ probability level. Statistical analyses were conducted by SPSS and MSTAT software.

3. RESULTS AND DISCUSSION

3.1. Yield

3.1.1. Grain yield

The amounts of applied irrigation water were 836, 1183, 1948, 1530 and 1256 mm for the sprinkler (1.0 ET_p) , sprinkler (1.5 ET_p) , continuous flooding, intermittent flooding (1-day interval) and intermittent flooding (2-day interval) treatments, respectively, in 2000. These values for 2001 were 971, 1374, 2262, 1779 and 1442 mm, respectively. The effect of the irrigation treatments on grain yield was significant for both of the experimental years (Tab. I). Maximum grain yields occurred

with continuous flooding and intermittent flooding in 2000 and 2001, respectively. Minimum grain yield was obtained with sprinkler irrigation treatments. The grain yield with continuous flooding was higher than that obtained for intermittent flooding in 2000, while it was higher than that for sprinkler irrigations. For both years, the difference between sprinkler irrigation and surface irrigation treatments was significant. However, significantly different results were only obtained with continuous and intermittent flooding treatments in 2000. For both years, the differences between sprinkler irrigation treatments were not significant.

The effect of nitrogen treatments on grain yield was significant in both years (Tab. I). The maximum value of yield was obtained with a maximum level of applied nitrogen. Nitrogen applications of 72 kg ha⁻¹ in 2000 and 92 kg ha⁻¹ in 2001 did not show a significant difference to the control treatment (32 kg N ha⁻¹). However, the treatments of 112 kg N ha⁻¹ in 2000 and 152 kg N ha⁻¹ in 2001 were significantly different from the 32, 72 and 92 kg N ha⁻¹ treatments. Similarly, Castillo et al. [6] reported that application of N fertilizer increased grain yield of rainfed lowland rice even when the rice crop was exposed to water deficit. Also, Zhong and Huang [27] indicated that grain yield and dry matter increased as the applied N rate was increased.

The grain yield differences in two consecutive years may not be related to the different plant populations (16 and 25 plants per m² in 2000 and 2001, respectively) (Tab. I) because there is not necessarily a large difference in panicle numbers; rather, differences in the unfilled grain percentage and 1000-grain weight were more obvious [19]. The optimum and low critical air temperatures during the flowering stage of rice are reported to be 30–33 and 22 °C, respectively [20]. The mean daily temperatures during this growth stage were 23.8 and 28.2 °C in 2000 and 2001, respectively. It is obvious that the mean daily temperature in 2000 was very close to the low critical air temperature for the flowering stage, and resulted in higher unfilled grains and lower grain yield in 2000.

	_	N tre	atmer	nt (kg ha ⁻¹)		
Year	Irrigation treatment	40		80		
	Sprinker (1*ETp)	9.47	d*	8.27	d	
	Sprinkler (1.5*ETp)	6.83	d	16.9	b	
2000	Continuous flooding	17.26	b	17.03	b	
	Intermittent fl.(1d. Int.)	12.98	С	18.45	b	
	Intermittent fl.(2d. Int.)	26.35	а	27.23	а	
		N tre	atmer	nt (kg ha ⁻¹)		
	-	60		120		
	Sprinker (1*ETp)	10.65	de	7.45	е	
	Sprinkler (1.5*ETp)	11.28	d	13.63	d	
2001	Continuous flooding	20.27	С	21.07	С	
	Intermittent fl.(1d. Int.)	18.66	С	21.91	С	
	Intermittent fl.(2d. Int.)	33.9	а	26.38	b	

Table II. Physiological efficiency (kg grain yield kg⁻¹ N absorbed) in different irrigation and nitrogen.

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

3.1.2. Straw yield

There was not a significant interaction effect between irrigation treatments and nitrogen application rates for straw yield. Furthermore, there was not a significant difference between the main effects of the irrigation treatments and nitrogen application rates (data were not shown). Similar results were obtained for both years of the experiment. The mean straw yields for the two consecutive years were 5284 and 5806 kg ha⁻¹, respectively. This difference is much smaller in comparison with grain yield differences, which indicates that the grain yield difference is not due to differences in the plant population in the two consecutive years.

3.2. N-use efficiency

3.2.1. Irrigation effects on N-use efficiency

The effect of irrigation treatments on physiological efficiency (PE) was statistically significant (Tab. II). The average maximum PE in 2000 (26.8 kg grain yield kg⁻¹ N absorbed) and 2001 (30.1 kg grain yield kg⁻¹ N absorbed) was obtained for intermittent flooding (2-day interval). The value of PE for this treatment was significantly higher than those for the other irrigation treatments. The average minimum PE was obtained in 2000 (8.9 kg grain yield kg⁻¹ N absorbed) and 2001 (9.1 kg grain yield kg⁻¹ N absorbed) for sprinkler irrigation (1.0 ET_p). The value of PE for this treatment was significantly lower than those for the other treatments except the sprinkler (1.5 ET_p) at 40 and 60 kg N ha⁻¹ applications. In both years, the difference between PE for continuous flooding and intermittent flooding (1-day interval) was not significant except for the 40 kg N ha⁻¹ application.

The effect of irrigation treatments on N-recovery efficiency (RE) was statistically significant (Tab. III). The average maximum RE, in 2000 (67%) and 2001 (64.4%), was obtained for

intermittent flooding (2-day interval) and intermittent flooding (1-day interval), respectively. The values of RE for these treatments were significantly higher than those for continuous and sprinkler irrigation treatments. The average minimum RE, in 2000 (53%) and 2001 (41%), was obtained for the sprinkler (1.0 ET_p) . The value of RE for this treatment was significantly lower than those for the other irrigation treatments, especially in 2001.

The effect of irrigation treatments on agronomic N-use efficiency (AE) was statistically significant (Tab. IV). The average maximum AE in 2000 (18.5 kg grain yield kg⁻¹ N applied) and 2001 (17.5 kg grain yield kg⁻¹ N applied) was obtained for intermittent flooding (2-day interval). The value of AE for this treatment was significantly higher than those for the other treatments. The average minimum AE was obtained in 2000 (4.6 kg grain yield kg⁻¹ N applied) and 2001 (3.2 kg grain yield kg⁻¹ N applied) for sprinkler irrigation (1.0 ET_p) with 40 and 60 kg N ha⁻¹ applications. The value of AE for this treatment was significantly lower than those for the other treatments except the sprinkler (1.5 ET_p). In both years, the difference between AE for continuous flooding and intermittent flooding (1-day interval) was not significant.

Timsina et al. [22] in their studies in Bangladesh reported that AE, PE and RE for rice ranged from 2.8 to 10.8, 5.2 to 27.5 and 33 to 61, respectively, and lower N-use efficiency in rice is attributed to larger losses of N from the soil-floodwater.

3.2.2. Nitrogen effects on N-use efficiency

The effect of nitrogen treatments on PE, RE and AE was not statistically significant for both years. However, the maximum values of these parameters were obtained for a high level of N application. These results were in accordance with Timsina et al. [22] who reported an increase in PE, RE and AE with increasing N application from 90 to 135 kg N ha⁻¹.

		N treatment (kg ha ⁻¹)					
Year	Irrigation treatment	40		80			
	Sprinker (1*ETp)	52	e*	53	е		
	Sprinkler (1.5*ETp)	63	abc	58	cde		
2000	Continuous flooding	52	е	55	de		
	Intermittent fl.(1d. Int.)	66	ab	6	bcd		
	Intermittent fl.(2d. Int.)	65	ab	69	а		
	N treatment (kg ha ⁻¹)						
	-	60		120			
	Sprinker (1*ETp)	33	g	5	ef		
	Sprinkler (1.5*ETp)	46	f	57	cd		
2001	Continuous flooding	53	de	57	cd		
	Intermittent fl.(1d. Int.)	61	bc	68	а		
	Intermittent fl.(2d. Int.)	55	cde	67	ab		

Table III. N-recovery efficiency (%) in different irrigation and nitrogen treatments for 2000 and 2001.

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

Table IV. Agronomic N-use efficiency (kg grain yield kg ⁻¹ N applied) in different	irrigation and nitro-
gen treatments for 2000 and 2001.	

		N tre	atme	N treatment (kg ha ⁻¹)				
Year	Irrigation treatment	40		80				
	Sprinker (1*ETp)	5.05	С*	4.13	С			
	Sprinkler (1.5*ETp)	4.51	С	9.16	b			
2000	Continuous flooding	8.68	b	9.39	b			
	Intermittent fl.(1d. Int.)	8.64	b	10.91	b			
	Intermittent fl.(2d. Int.)	18.19	а	18.76	а			
		N tre	atme	ent (kg ha ⁻¹)				
		60		120				
	Sprinker (1*ETp)	2.98	е	3.38	е			
	Sprinkler (1.5*ETp)	4.17	е	7.64	d			
2001	Continuous flooding	10.7	С	13.07	bc			
	Intermittent fl.(1d. Int.)	11.85	С	14.52	b			
	Intermittent fl.(2d. Int.)	18.13	а	16.9	а			

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

3.2.3. Interaction effects on N-use efficiency

Interaction effects between irrigation and nitrogen treatments on PE were statistically significant in both years (Tab. II). In 2000, maximum PE was obtained for intermittent flooding (2-day interval) with 40 and 80 kg N ha⁻¹, while in 2001, this value was obtained for intermittent flooding (2-day interval) only with 60 kg N ha⁻¹. In both years, the values of PE for sprinkler treatment (1.0 ET_p) were statistically smaller than those for sprinkler treatment (1.5 ET_p) at a higher N application rate (80 and 120 kg N ha⁻¹), while this treatment was

not significantly different from the sprinkler (1.5 ET_{p}) at a lower N application rate (40 and 60 kg N ha⁻¹).

Interaction effects between irrigation and nitrogen treatments on RE were statistically significant in both years (Tab. III). In 2000, maximum RE was obtained for intermittent flooding (2-day interval) with 80 kg N ha⁻¹, while this treatment was not significantly different from intermittent flooding with 40 kg N ha⁻¹ and the sprinkler (1.5 ET_p) with 40 kg N ha⁻¹. Minimum RE was obtained in continuous flooding with 40 kg N ha⁻¹, while this treatment was not significantly different from

			N tr	eatment (k	g ha ⁻¹)		
Year	Irrigation treatment	0		40		80	
	Sprinker (1*ETp)	6.28	h*	8.01	ef	10.44	а
	Sprinkler (1.5*ETp)	6.61	gh	8.44	de	10.35	а
2000	Continuous flooding	4.97	I	7.41	fg	9.19	bcd
	Intermittent fl.(1d. Int.)	6.53	h	8.78	cde	9.88	ab
	Intermittent fl.(2d. Int.)	5.77	h	7.44	fg	9.45	bc
			N tr	eatment (k	g ha ⁻¹)		
		0		60		120	
	Sprinker (1*ETp)	7.56	fg	8.54	cde	12.1	а
	Sprinkler (1.5*ETp)	7.69	fg	9.18	С	10.42	b
2001	Continuous flooding	6.61	h	8.08	def	8.8	cd
	Intermittent fl.(1d. Int.)	7.24	gh	8.52	cde	10.57	b
	Intermittent fl.(2d. Int.)	6.68	h	7.89	efg	9.94	b

Table V. Grain protein (%) in different irrigation and nitrogen treatments for 2000 and 2001.

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

sprinkler (1.0 ET_p) treatments, the sprinkler (1.5 ET_p) with 80 kg N ha⁻¹ and continuous flooding with 80 kg N ha⁻¹. In 2001, maximum RE was obtained for intermittent flooding (1-day interval) with 120 kg N ha⁻¹, while this treatment was not significantly different from intermittent flooding (2-day interval) with 120 kg N ha⁻¹. Minimum RE was obtained for the sprinkler (1.0 ET_p) with 60 kg N ha⁻¹, and this treatment was significantly different from the other treatments.

Interaction effects between irrigation and nitrogen treatments on AE were statistically significant in both years (Tab. IV). In 2000, maximum AE was obtained for intermittent flooding (2-day interval) with 80 kg N ha⁻¹, while this treatment was not significantly different from intermittent flooding (2-day interval) with 40 kg N ha⁻¹. Minimum AE was obtained for the sprinkler (1.0 ET_p) with 80 kg N ha⁻¹, while this treatment was not significantly different from sprinkler treatments (1.0 and 1.5 ET_p) with 40 kg N ha⁻¹. In 2001, maximum AE was obtained for intermittent flooding (2-day interval) with 60 kg N ha⁻¹, while this treatment was not significantly different from sprinkler (1.0 ET_p) with 60 kg N ha⁻¹, while this treatment was not significantly different from intermittent flooding (2-day interval) with 60 kg N ha⁻¹. Minimum RE was obtained for the sprinkler (1.0 ET_p) with 60 kg N ha⁻¹, while this treatment was not significantly different from the sprinkler (1.0 ET_p) with 120 kg N ha⁻¹ and the sprinkler (1.5 ET_p) with 60 kg N ha⁻¹.

3.3. Grain protein

Multiple regression between the grain protein, N application rates and applied irrigation water resulted in the following relationship:

$$GP = 6.92(\pm 0.50) + 0.0343(\pm 0.0029)N - 0.001(\pm 0.0003)W (1)$$

R² = 0.84, SE = 0.68, n = 30, P = 1.45 × 10⁻¹¹

where GP is grain protein in %, N is the nitrogen application rate in kg ha⁻¹, and W is applied irrigation water in mm. The amounts of applied irrigation water were reported by Pirmoradian et al. [19]. The regression coefficients indicate that with increasing applied N, grain protein was increased, and with increasing applied water, grain protein was decreased. This might be due to the fact that with higher applied water in continuous flooding or intermittent irrigation with lower intervals, nitrogen leaching in the soil might have resulted in lower nitrogen uptake by the plant and lower grain protein concentration in the grain.

3.3.1. Irrigation effects

The effect of irrigation treatments on the grain protein was statistically significant (Tab. V). In 2000, the average maximum grain protein (8.5%) was obtained for the sprinkler (1.5 ET_{p}), while this treatment was not significantly different from the sprinkler (1.0 ET_{p}) and intermittent flooding (1-day interval). The average minimum grain protein (7.1%) was obtained for continuous flooding and it was not significantly different from intermittent flooding (2-day interval). In 2001, the average maximum grain protein (9.4%) was obtained for the sprinkler (1.0 ET_{p}), while this treatment was not significantly different from the sprinkler (1.5 ET_{p}) and intermittent flooding (1-day interval). In 2001, the average maximum grain protein (9.4%) was obtained for the sprinkler (1.0 ET_{p}), while this treatment was not significantly different from the sprinkler (1.5 ET_{p}) and intermittent flooding (1-day interval). The average minimum protein concentration of grain (7.8%) was obtained for continuous flooding and it was not significantly different from that obtained for intermittent flooding (2-day interval).

3.3.2. Nitrogen effects on grain protein

The effect of nitrogen treatments on the grain protein was statistically significant (Tab. V). In both years, the average maximum and minimum grain proteins (9.9% and 6.0% for 2000 and 10.4% and 7.2% for 2001) were obtained for the highest and lowest levels of N application, respectively. In other words, increasing N application resulted in higher grain protein.

3.3.3. Interaction effects on grain protein

Interaction effects between irrigation and nitrogen treatments on grain protein were statistically significant in both

			I	N treatment (kg ha ⁻¹)		
Year	Irrigation treatment	0		40	80	
	Sprinker (1*ETp)	0.33	e*	0.6 d	0.98	а
	Sprinkler (1.5*ETp)	0.33	е	0.62 d	0.92	а
2000	Continuous flooding	0.095	f	0.38 e	0.58	d
	Intermittent fl.(1d. Int.)	0.35	е	0.65 cd	0.79	b
	Intermittent fl.(2d. Int.)	0.14	f	0.4 e	0.71	С
			I	N treatment (kg ha ⁻¹)		
		0		60	120	
	Sprinker (1*ETp)	0.42	ef	0.53 cd	1.16	а
	Sprinkler (1.5*ETp)	0.41	ef	0.56 c	0.81	b
2001	Continuous flooding	0.29	g	0.35 fg	0.54	cd
	Intermittent fl.(1d. Int.)	0.34	fg	0.53 cd	0.86	b
	Intermittent fl.(2d. Int.)	0.38	ef	0.46 de	0.8	b

Table VI. Straw nitrogen concentration (%) in different irrigation and nitrogen treatments for 2000 and 2001.

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

years (Tab. V). In 2000, maximum grain protein was obtained for the sprinkler (1.0 ET_p) with 80 kg N ha⁻¹, while this treatment was not significantly different from the sprinkler (1.5 ET_p) and intermittent flooding (1-day interval) with 80 kg N ha⁻¹. Minimum grain protein was obtained in continuous flooding with no N application, while this treatment's result was significantly smaller than those obtained for the other treatments. In 2001, maximum grain protein was obtained for the sprinkler (1.0 ET_p) with 120 kg N ha⁻¹, while it was significantly higher than those obtained for the other treatments. Minimum grain protein was obtained for continuous flooding with no N application, while this treatment was not significantly different from intermittent flooding treatments with no N application.

3.4. Straw nitrogen concentration

3.4.1. Irrigation effects on straw nitrogen concentration

The effect of irrigation treatments on straw nitrogen concentration was statistically significant (Tab. VI). In 2000, the average maximum nitrogen of straw (0.63%) was obtained for the sprinkler (1.0 ET_{p}), while this treatment was not significantly different from the sprinkler (1.5 ET_{p}) and intermittent flooding (1-day interval). The average minimum straw nitrogen concentration (0.35%) was obtained for continuous flooding and it was not significantly different from that obtained for intermittent flooding (2-day interval). In 2001, the average maximum nitrogen concentration of straw (0.70%) was obtained for the sprinkler (1.0 ET_{p}) and it was significantly higher than those obtained for the other treatments. The average minimum straw nitrogen concentration (0.39%) was obtained for continuous flooding and it was not significantly higher than those obtained for the other treatments. The average minimum straw nitrogen concentration (0.39%) was obtained for continuous flooding and it was significantly lower than those obtained for the other treatments.

3.4.2. Nitrogen effects on straw nitrogen concentration

The effect of nitrogen treatments on straw nitrogen concentration was statistically significant (Tab. VI). In both years, the average maximum and minimum straw nitrogen concentrations (0.80% and 0.25% for 2000 and 0.83% and 0.36% for 2001) were obtained for the highest and lowest levels of N application, respectively. In other words, increasing N application resulted in increasing straw nitrogen concentration.

3.4.3. Interaction effects on straw nitrogen concentration

Interaction effects between irrigation and nitrogen treatments on straw nitrogen concentration were statistically significant in both years (Tab. VI). In 2000, maximum straw nitrogen concentration was obtained for the sprinkler (1.0 ET_n) with 80 kg N ha⁻¹, while this treatment was not significantly different from the sprinkler (1.5 ET_{p}) with 80 kg N ha⁻¹. Minimum straw nitrogen concentration was obtained for continuous flooding with no N application, while this treatment was not significantly different from intermittent flooding (2-day interval). In 2001, maximum straw nitrogen concentration was obtained for the sprinkler (1.0 ET_p) with 120 kg N ha⁻¹, while it was significantly higher than those obtained for the other treatments. Minimum straw nitrogen concentration was obtained for continuous flooding with no N application, while this treatment was not significantly different from continuous flooding with 60 kg N ha⁻¹ and intermittent flooding (1-day interval) with no N application.

3.5. Nitrogen uptake

Nitrogen uptakes by grain and straw (kg ha⁻¹) were calculated by multiplication of grain and straw nitrogen concentrations (kg N absorbed kg⁻¹ grain yield) by grain and straw yields (kg ha⁻¹). Total plant nitrogen uptake was obtained by summing the grain and straw N uptake. The results are shown in Table VII. The effect of irrigation and nitrogen treatments and their interaction effects on nitrogen uptake were statistically significant. In 2000, maximum and minimum of N uptake were obtained for intermittent flooding (1-day interval) with 80 kg N ha⁻¹ and intermittent flooding (2-day interval) without N

			N	treatment (k	g ha ⁻¹)		
Year	Irrigation treatment	0		40		80	
	Sprinker (1*ETp)	27.7	f*	50.4	de	82.7	b
	Sprinkler (1.5*ETp)	28	f	49.6	е	82.3	b
2000	Continuous flooding	31.2	f	58.6	cd	92.7	а
	Intermittent fl.(1d. Int.)	41.8	е	60.5	С	94.1	а
	Intermittent fl.(2d. Int.)	23.15	f	48.3	е	91.6	а

Table VII. Total plant nitrogen uptake (kg ha⁻¹) in different irrigation and nitrogen treatments for 2000 and 2001.

			l	N treatment (kg ha ⁻¹)		
		0		60	120	•
	Sprinker (1*ETp)	67.8	fg	78.5 ef	118.5	b
	Sprinkler (1.5*ETp)	60.4	g	81.6 ef	122.1	b
2001	Continuous flooding	59.7	g	86.4 de	123.1	b
	Intermittent fl.(1d. Int.)	70.2	fg	96.3 cd	141.1	а
	Intermittent fl.(2d. Int.)	72.3	fg	101.8 c	148.4	а

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

Table VIII. SPAD readings in 2001.

Time of			N	treatment (kg	g ha ⁻¹)		
SPAD reading	Irrigation treatment	0		60		120	
	Sprinker (1*ETp)	34.1	ef*	35.5	cd	37.2	ab
Before N	Sprinkler (1.5*ETp)	34.9	de	36.2	bc	37.7	а
application	Continuous flooding	31.9	h	32.7	gh	33.6	fg
	Intermittent fl.(1d. Int.)	33.2	fg	35.5	cd	37	ab
	Intermittent fl.(2d. Int.)	34.9	de	36.9	ab	37.7	а
	Sprinker (1*ETp)	36.1	*	38.1	fgh	40.9	abc
After N	Sprinkler (1.5*ETp)	38	gh	40.2	bcd	41.9	ab
application	Continuous flooding	34.6	j	38.4	efgh	39.7	cdef
	Intermittent fl.(1d. Int.)	36.9	ĥi	39.9	cde	41.7	ab
	Intermittent fl.(2d. Int.)	38.9	defg	40.8	abc	42.3	а

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

application, respectively. These values for 2001 were obtained for intermittent flooding (2-day interval) with 120 kg N ha⁻¹ and continuous flooding without N application, respectively. The N uptake for treatments without N application could be absorbed from soil nitrogen or phosphate ammonium fertilizer that was applied for all treatments (32 kg N ha^{-1}). In 2000, the N uptake for treatments without N application was lower than those values for 2001. These differences may be related to lower grain yield in this year due to lower air temperature during the flowering period, which resulted in higher unfilled grains. Therefore, the higher unfilled grain in the panicle resulted in lower N uptake.

Multiple regression between total nitrogen uptake, grain yields, N application rates and applied irrigation water resulted in the following relationship:

$$NU = 9.89(\pm 5.99) + 0.0136(\pm 0.0016)Y + 0.471(\pm 0.041)N - 0.0134(\pm 0.0047)W$$
(2)

$$R^2 = 0.94$$
, SE = 8.12, n = 30, P = 1.42×10^{-16}

where NU is total plant nitrogen uptake in kg ha⁻¹, Y is grain yield in kg ha⁻¹, N is nitrogen application rate in kg ha⁻¹ and W is applied irrigation water in mm. The regression coefficients indicate that with increasing grain yield and applied N, nitrogen uptake was increased, and with increasing applied water, nitrogen uptake was decreased. This reduction in nitrogen uptake could be due to the effect of nitrate leaching in the root zone at higher applied water in continuous and intermittent flooding with a short interval.

3.6. SPAD chlorophyll concentration

SPAD readings in 2001, before and after the second N application, are shown in Table VIII. The effects of irrigation and nitrogen treatments on leaf chlorophyll concentration (SPAD readings) were statistically significant (Tab. VIII). In irrigation treatments, before N application, the minimum SPAD reading (32.8 in continuous flooding) was significantly lower than those for the other treatments, while after N application, this value (37.6) was not significantly different from the sprinkler (1.0 ET_p) and intermittent flooding (1-day interval). In fact, the differences between SPAD readings before N application were greater than those after N application. The difference between the average maximum and minimum SPAD readings, before N application, was 3.7, while this value for after N application was 3.1. These results indicate that continuous flooding might have leached the soil nitrogen with more severity.

In N treatments, before N application, with increasing applied N, the SPAD reading was significantly increased. After N application, the average maximum SPAD reading was obtained for 120 kg N ha⁻¹, while it was not significantly different from that obtained for 60 kg N ha⁻¹.

Interaction effects of irrigation and nitrogen treatments on SPAD readings, before N application, were statistically significant, while those effects after N application were not statistically significant (Tab. VIII). Before N application, the maximum SPAD reading was obtained for the sprinkler (1.5 ET_p) with 120 kg N ha⁻¹ and it was not significantly different from those obtained for intermittent flooding (2-day interval) with 60 and 120 kg N ha⁻¹, intermittent flooding (1-day interval) with 120 kg N ha⁻¹ and the sprinkler (1.0 ET_p) with 120 kg N ha⁻¹.

Plant access to nitrogen and increasing leaf chlorophyll resulted in differences between SPAD readings before (35) and after (39) N application. In irrigation treatments, the minimum (2.9) and maximum (4.8) difference between SPAD readings before and after N applications were obtained for the sprinkler (1.0 ETp) and continuous flooding, respectively. Also, minimum and maximum SPAD readings before and after N applications were obtained for continuous flooding and intermittent flooding (2-day interval), respectively. These results indicate that the maximum nitrate leaching might have happened in continuous flooding. With increasing N application, SPAD readings before and after N applications, and their differences, were increased.

A linear relationship between SPAD readings and leaf nitrogen concentration (%) before and after the second N application (in the middle of the growing season) was obtained as follows (Fig. 1):

LNC =
$$0.081(SR - 9.4)$$
,
R² = 0.87, SE = 0.094, n = 30, P = 4.9×10^{-13} (3)

where LNC is leaf nitrogen concentration (%) and SR is SPAD readings. This equation indicates that chlorophyll concentration was positively correlated with leaf N concentration in the middle of the growing season. However, a threshold SR of 9.4 was required to begin to show the leaf nitrogen concentration. Therefore, SPAD readings can be used to distinguish N status and the effect of different N treatments on yield. However, equation (3) may be different for different growth stages because specific leaf area (SLA) changes dramatically from emergence to flowering.

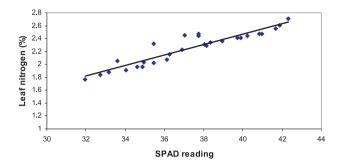


Figure 1. Relationship between SPAD readings and leaf nitrogen concentration (%), Y = 0.081(X - 9.4), $R^2 = 0.87$.

3.7. Soil nitrogen

Soil nitrogen (N-NH₄ and N-NO₃) was measured at depths of 0–30 and 30–60 cm. The soil nitrogen contents at these depths were not statistically significant. Therefore, the soil nitrogen content in the root zone (45 cm) was obtained by the summing of soil nitrogen at a depth of 0–30 cm and one-half of soil nitrogen at a depth of 30–60 cm (Tabs. IX, X).

3.7.1. Irrigation treatment effects on soil nitrogen

Irrigation effects on soil nitrogen before the second N application and at the end of the growing season were statistically significant for both years (Tabs. IX, X). For all cases, the average maximum soil nitrogen was obtained for intermittent flooding (2-day interval). This treatment was not statistically different from sprinkler irrigation treatments and intermittent flooding (1-day interval). The average minimum soil nitrogen was obtained for continuous flooding and this treatment was statistically different from those obtained in the other treatments. Therefore, maximum nitrogen leaching occurred in continuous flooding irrigation.

3.7.2. Nitrogen treatment effects on soil nitrogen

The nitrogen treatment effect on soil nitrogen before the second N application and at the end of the growing season were statistically significant for both years (Tabs. IX, X). For all cases, the average maximum and minimum soil nitrogen contents were obtained for the highest and the lowest second N application rates, respectively. Soil nitrogen contents for all N application rates were statistically different. They increased as the N application rates increased.

3.7.3. Interaction effects

Interaction effects of irrigation treatments and nitrogen application rates on soil nitrogen content before the second N application and at the end of the growing season were statistically significant for both years (Tabs. IX, X). In 2000, before the second N application, maximum soil nitrogen was obtained for the sprinkler (1.0 ET_p) with 112 kg N ha⁻¹. This treatment was not significantly different from the sprinkler (1.5 ET_p) and intermittent flooding (2-day interval) with 112 kg N ha⁻¹. At the end of the growing season, maximum soil nitrogen content was

Time of			Ν	treatment (kg ha ⁻¹)		
measurement	Irrigation treatment	32		72	112	
	Sprinker (1*ETp)	13.3	ef*	41.5 c	94	а
Before second	Sprinkler (1.5*ETp)	19.3	ef	37.4 cd	85.2	ab
N application	Continuous flooding	6	f	23.9 de	52.4	С
	Intermittent fl.(1d. Int.)	17.3	ef	15.3 ef	74.8	b
	Intermittent fl.(2d. Int.)	17.3	ef	41.5 c	89.6	ab
	Sprinker (1*ETp)	13.3	de	43.6 e	87.7	а
At the end of	Sprinkler (1.5*ETp)	23.3	cd	43.5 b	76.9	а
growth season	Continuous flooding	8	е	28 c	48.4	b
	Intermittent fl.(1d. Int.)	21.3	cde	25.4 cd	76.9	а
	Intermittent fl.(2d. Int.)	21.3	cde	47.7 b	85.4	а

Table IX. Soil nitrogen (N-NH₄ and N-NO₃) for different treatments in 2000.

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

Table X. Soil nitrogen (N-NH₄ and N-NO₃) for different treatments in 2001.

Time of			Ν	l treatment (kg ha ⁻¹)		
measurement	Irrigation treatment	32		92	152	
	Sprinker (1*ETp)	14.7	fg*	37.4 d	53.9	bc
Before second	Sprinkler (1.5*ETp)	13.3	fg	35.4 de	60.1	b
N application	Continuous flooding	4	g	23.9 ef	36.1	de
	Intermittent fl.(1d. Int.)	19.3	f	41.5 d	62.2	ab
	Intermittent fl.(2d. Int.)	17.3	f	47.7 cd	72.7	а
	Sprinker (1*ETp)	13.9	fg	37.5 cd	62.2	b
At the end of	Sprinkler (1.5*ETp)	13.5	fg	31.5 cd	65.1	ab
growth season	Continuous flooding	6.4	g	20.1 ef	38.2	cd
	Intermittent fl.(1d. Int.)	17.5	fg	29.6 de	64.2	ab
	Intermittent fl.(2d. Int.)	13.5	fg	41.6 c	74.6	а

* Means followed by the same letters in each column are not significantly different at 5% level of probability.

obtained for the sprinkler (1.0 ET_p) with 112 kg N ha⁻¹. This treatment was not significantly different from the sprinkler (1.5 ET_p) , and intermittent flooding treatments (1-day interval and 2-day interval) with 112 kg N ha⁻¹.

In 2001, before the second N application, maximum soil nitrogen content was obtained for intermittent flooding (2-day interval) with 152 kg N ha⁻¹. This treatment was not significantly different from intermittent flooding (1-day interval) with 152 kg N ha⁻¹. At the end of the growing season, maximum soil nitrogen content was obtained for intermittent flooding (2-day interval) with 152 kg N ha⁻¹. This treatment was not significantly different from the sprinkler (1.5 ET_p) and intermittent flooding (1-day interval) with 112 kg N ha⁻¹.

For all cases and N application rates, minimum soil nitrogen content was obtained in continuous flooding. Therefore, maximum nitrogen leaching (about 50%) occurred in continuous flooding irrigation.

4. CONCLUSION

The results indicated that low and high applied water affected the plant and soil factors in N uptake and decreased N-use efficiency for rice. Therefore, optimum applied water was obtained in intermittent flooding (2-day interval). Reduction in nitrogen uptake at high applied water in continuous flooding irrigation could be due to the effect of nitrate leaching in the root zone. The reduction in N uptake at low applied water in sprinkler irrigation treatments could be due to the inability of the roots to absorb N and translocate it to the plant top.

With respect to the relationship between N uptake and grain protein and leaf chlorophyll, these parameters can also be affected by applied water and N application. Appropriate linear models were proposed to show these relationships.

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