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The Effect of Irrigation Management and Nitrogen Fertilizer On Grain Yield and Water-use Efficiency of Rice Cultivars in Northern Iran

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Abstract
Alternate wetting and drying irrigation (AWDI) is a water-conserving strategy in rice fields. An experiment, conducted as a split-split plot design and based on a randomized complete block design with three repetitions, investigated the effect of intermittent irrigation and nitrogen on yield and water-use efficiency during 2017 and 2018 crop years in northern Iran. The irrigation intervals (flooding (I1), 7 days (I2), 14 days (I3)) were main factors, different levels of nitrogen fertilizer (50 kg/ha (N1), 75 kg/ha (N2), 100 kg/ha (N3)) were sub-factors, and cultivars (Gilaneh (C1) and Hashemi (C2)) were sub-sub-factors. Results showed that compared to flood irrigation, intermittent irrigation led to 16 to 43% and 13 to 43% water economization in 2017 and 2018, respectively, accompanied by a significant increase in water-use efficiency. By increasing the irrigation intervals, grain yield of the Gilaneh cultivar decreased significantly in all three fertilization levels. Compared to flood irrigation, total dry matter reduced significantly by increasing the irrigation interval. Maximum grain yield, total dry matter, and harvest index were achieved for the 100 kg/ha nitrogen fertilizer treatment in I1, I2, and I3 levels. Intermittent irrigation reduced leaf relative water content and increased leaf proline content in both cultivars. Leaf relative water content (R = 0.89**) showed the highest correlation coefficient with grain yield. The Hashemi cultivar showed higher leaf relative water content and leaf proline content and, while achieving 16.61% and 13.94% water conservation in the I2 treatment in 2017 and 2018, respectively, resulted in a yield equivalent to flood irrigation.

Keywords Irrigation · Nitrogen · Proline · Relative water content · Water use efficiency

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Der Effekt von Bewässerungsmanagement und Stickstoffdünger auf Kornertrag und Wassernutzungseffizienz von Reissorten im nördlichen Iran

Zusammenfassung

Die intermittierende Bewässerung (*alternate wetting and drying irrigation*, AWDI) ist eine wassersparende Strategie in Reisfeldern. Ein Experiment, das als Split-Split-Plot-Design durchgeführt wurde und auf einem randomisierten vollständigen Blockdesign mit drei Wiederholungen basiert, untersuchte die Wirkung von intermittierender Bewässerung und Stickstoff auf den Ertrag und die Wassernutzungseffizienz in den Erntejahren 2017 und 2018 im nördlichen Iran. Die Bewässerungsintervalle (Überflutung (I1), 7 Tage (I2), 14 Tage (I3)) waren Hauptfaktoren, verschiedene Stickstoffdüngermengen (50 kg/ha (N1), 75 kg/ha (N2), 100 kg/ha (N3)) waren Subfaktoren und die Kultursorten (Gilaneh (C1) und Hashemi (C2)) waren Sub-Subfaktoren. Die Ergebnisse zeigten, dass die intermittierende Bewässerung im Vergleich zur Flutbewässerung zu einer Wassereinsparung von 16 bis 43 % bzw. 13 bis 43 % in den Jahren 2017 und 2018 führte, begleitet von einer signifikanten Steigerung der Wassernutzungseffizienz. Durch die Erhöhung der Bewässerungsintervalle sank der Kornertrag der Sorte Gilaneh in allen drei Düngungsstufen signifikant. Im Vergleich zur Flutbewässerung verringerte sich die Gesamttrockenmasse durch die Erhöhung des Bewässerungsintervalls signifikant. Der maximale Kornertrag, die Gesamttrockenmasse und der Ernte-Index wurden bei der Behandlung mit 100 kg/ha Stickstoffdünger in den Stufen I1, I2 und I3 erreicht. Die intermittierende Bewässerung reduzierte den relativen Wassergehalt der Blätter und erhöhte den Prolingehalt der Blätter in beiden Sorten. Der relative Wassergehalt der Blätter (R = 0,89**) zeigte den höchsten Korrelationskoeffizienten mit dem Kornertrag. Die Sorte Hashemi zeigte einen höheren relativen Blatt-Wasser-Gehalt und einen höheren Blatt-Prolin-Gehalt und erreichte bei der I2-Behandlung in den Jahren 2017 und 2018 eine Wassereinsparung von 16,61 % bzw. 13,94 %, was zu einem Ertrag führte, der der Flutbewässerung entsprach.

Schlüsselwörter Bewässerung · Stickstoff · Prolin · Relativer Wassergehalt · Wassernutzungseffizienz

Introduction

Rice is the staple food of many countries around the world, providing an average of 50 to 80% of the daily caloric needs of over three billion people (Khush 2005). The water requirement of rice during its entire growth period is higher than other field crops, consuming about 80% of the fresh irrigation water in Asia. However, the water resources available to agriculture are decreasing due to domestic and industrial consumptions, and the need for production is rising due to increased demand and population growth. Therefore, increased yield and less, more efficient consumption of the available water is a necessity for the rice-growing regions (Bouman and Tuong 2001). The alternate wetting and drying irrigation (AWDI) in the field is a water-saving regime that has not been universally accepted due to its potential for yield reduction (Carrijo et al. 2017). In this method, the soil is flooded and non-flooded in alternate periods during the growing season which leads to the reduced entrance of water to the rice fields, thus increasing water-use efficiency (Wang et al. 2016). Reduced water consumption in this method must not put yield at risk. The results of research studies regarding the impact of this method on yield are still unclear (Wang et al. 2016; Liu et al. 2013; Bouman and Tuong 2001), while other studies such as Rahman and Bulbul (2014) in Bangladesh and Tan et al. (2013) in China indicate that AWDI has been successful in conserving water without causing a significant reduction in yield.

It has been reported that AWDI reduces yield by an average of 5.4%. However, if applied with slight intensity (when soil water potential is equal to –20 kPa or the water level isn't less than 15 cm from the soil surface), in most situations, yield doesn't show a significant reduction. Contrastingly, in the case of intense AWDI (when soil is dry at less than –20 kPa) yield could reduce up to 22.6% in comparison to flood irrigation. This decline in yield is more likely in soils with a pH of equal or higher than seven and less than 1% carbon, or in cases where AWDI occurs throughout the plant's growing season (Carrijo et al. 2017).

On the other hand, nitrogen is the most important element in rice cultivation and the increased application of this fertilizer is the reason for the advanced yield of rice in the past fifty years (Peng et al. 2010). Nitrogen increases the growth and yield of crop plants through its effect on photosynthesis. RuBisCO and other mesophyll proteins comprise about 75% of the total nitrogen content of cells and in the case of nitrogen deficiency, the performance of these key proteins is impeded during the photosynthesis process (Evans 1989). Improved growth caused by nitrogen fertilizers leads to better canopy coverage while increasing transpiration and reducing water evaporation from the soil surface. The improved growth of aerial parts also leads to advanced root growth, thus providing better access to available water resources (Haefele et al. 2016). Surely, compatible nitrogen osmolytes such as proline and other amino acids that play a role in saving plant-cell water content

Table 1 Meteorological information of the experimental site during the growing season of rice

Year	Month	Minimum temp. (°C)	Maximum temp. (°C)	Average temp. (°C)	Rainfall (mm)	Sunny hour (day-1)
2017	Apr	8.5	18.6	13.58	86.2	140
	May	14.19	24.2	19.19	27.8	168.8
	Jun	18.77	28.13	23.45	18.6	229.5
	Jul	20.66	31.6	25.9	13.8	232.5
	Aug	22.1	33.8	28	0	293.7
	Sep	21.3	32.5	26.9	61	245.8
2018	Apr	8.59	18.77	13.6	20.4	145.9
	May	14.2	24.5	19.4	37.2	170.4
	Jun	18.2	27.9	23	48.7	230.3
	Jul	22.913	33.26	28.08	30.8	295.4
	Aug	22.72	31.28	27	68.4	164.9
	Sep	19.73	30.44	25.08	13.8	209.7

Table 2 Physical and chemical soil properties of the experimental site

Year	Absorbable potash (ppm)	Absorbable phosphorus (ppm)	Total nitrogen in soil (%)	Soil pH	Electrical conductivity (dS/m)	Soil type	Sp
2017	280	17.8	0.184	7.4	1.2	Si-Cl	75
2018	290	17	0.155	7.4	1.12		

during osmotic stress must also be considered in this path (Zhong et al. 2017). In AWDI systems, the soil becomes dry and is wetted again and this process is repeated many times which can affect the availability of minerals such as nitrogen (Song et al. 2019). In AWD irrigation systems, plants with relatively high nitrogen availability exhibit better growth as compared to plants with lower nitrogen content. A moisture regime in which irrigation intervals are close to each other and mild, applying an optimum amount of N fertilizer results in the highest level of grain yield and water-use efficiency. Also, an irrigation regime in which frequent irrigation intervals had led to severe drought, increased N fertilizer application improved the reduced yield caused by drought stress to some extent (Wang et al. 2016). Ashouri (2014) stated that by implementing 5 and 8-day irrigation intervals, grain yield, leaf relative water content, and water-use efficiency will be similar to flood irrigation and the cultivation of hybrid rice in northern Iran doesn't require flood irrigation when by implementing 8-day irrigation intervals and applying 120 kg/ha N fertilizer, yield similar to flood irrigation can be achieved while consuming less water.

These water-saving systems (AWDI) may exhibit different results in various regions, due to the differences in climate, soil properties, and cultivar genetics. This requires regional studies in order to obtain useful recommendations for the farmers of each region. Studies regarding AWDI in rice fields of northern Iran and its interaction with N fertilizer application and cultivar are limited and considering the increased limitation of water availability in this region, the necessity of conducting research in this field is felt more than ever. The present research was designed and executed with the aim of investigating the effect of the AWD irrigation system on rice cultivation in northern Iran and studying its interaction with nitrogen fertilizer on rice yield attributes and yield-dependent physiological attributes of two rice cultivars in this region.

Materials and Methods

The present study was conducted in the Rice Research Institute of Rasht, Iran during the 2017 and 2018 crop years. The meteorological information of the target region during the experimental period is depicted in Table 1. The average temperature was similar during both years of experimentation, but average rainfall in 2018 was slightly higher than in 2017 (Table 2). Before performing the experiment, the physical and chemical soil properties of the experimental site were measured in the Department of Water and Soil laboratory of the aforementioned institute, the results of which are depicted in Table 2.

The experiment was carried out as a split-split plot design based on a randomized complete block design with three repetitions. The irrigation intervals, i.e. flooding (I1), 7 days (I2), 14 days (I3), were determined as main factors, the different levels of nitrogen fertilizer, i.e. 50 kg/ha (N1), 75 kg/ha (N2), 100 kg/ha (N3), were considered as sub-factors, and the improved Gilaneh cultivar (C1) and native, wild Hashemi cultivar were sub-sub factors. The preparation of land via winter tillage was carried out in both years of research. After disinfecting seeds in the nursery with a 2:1000 ratio of Carboxin-Thiram fungicide, broadcasting seeds was carried out in March/April. The preparation of the growing bed was carried out in early April. After reaching the approximate height of 30 cm, the seedlings were planted at a density of 16 plants per square meter in the main field. Seedlings were planted manually. The experimental units were 9 m^2 (plot dimension of 3 × 3). Post-cultivation application of Butachlor herbicide (3 L/ha) and manual weeding were carried out for weed control. Trichogramma wasps were used to biologically control the Asiatic rice borer, *Chilo suppressalis*. To apply the I2 and I3 irrigation treatments, the plots were kept flooded thirty days after planting the seedlings, and then these plots were irrigated consecutively in 7 and 14-day intervals, respectively, whereas the I1 plot was kept flooded throughout the entire growing season. The volume of water used in the three irrigation regimes was recorded via a counter. Nitrogen fertilizer (46% nitro-

Table 3 Analysis of variance of the effect of irrigation interval and nitrogen fertilizer on the studied characteristic in two cultivars in 2017 and 2018

S.O.V	Df	Yield		Dry matter		HI		RWC		WUE		Proline	
		2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
R	2	0.005	0.003	0.01	0.06	0.88	2.16	2.12	2.8	0.0002	0.0005	0.05	0.19
Irrigation (a)	2	1.72**[a]	1.2**	14.6**	2.1**	12.5**	18.97^{ns}	573.6**	529.8**	0.21**	0.286**	74.98**	75.8**
Error a	4	0.007^{ns}	0.002^{ns}	0.04^{ns}	0.009^{ns}	2.1^{ns}	0.19^{ns}	9.43^{ns}	3.4^{ns}	0.0004^{ns}	0.0006^{ns}	0.19^{ns}	0.98*
N fertilizer (b)	2	10.2**	10.12**	21.2**	14.0**	106.9 **	135.1**	1935.8**	1792.4**	0.51**	0.536**	73.27**	80.6**
a × b	4	0.087**	0.14**	0.37**	0.17^{ns}	33.3**	19.3^{ns}	17.5*	12.0*	0.02**	0.026**	33.3**	31.05*
Error b	12	0.009^{ns}	0.001^{ns}	0.019^{ns}	0.03^{ns}	3.6^{ns}	6.4^{ns}	1.97^{ns}	2.4^{ns}	0.0009^{ns}	0.0004^{ns}	0.48^{ns}	0.3^{ns}
Cultivar (c)	1	0.008^{ns}	0.02^{ns}	6.21**	3.02**	168.5**	294.0**	74.6**	156.6**	0.07**	0.08**	4.59**	15.4**
a × c	2	0.64**	0.08**	0.03^{ns}	0.25*	148.4**	15.97^{ns}	1.8^{ns}	1.1^{ns}	0.01**	0.0001^{ns}	0.12^{ns}	0.02^{ns}
b × c	2	0.74**	0.95**	0.05^{ns}	1.23**	161.1**	87.9**	0.68^{ns}	38.4**	0.04**	0.064**	1.96*	3.7**
a × b × c	4	0.09**	0.19**	0.036**	0.77**	43.9**	30.7*	15.0*	14.7**	0.009**	0.019**	1.46*	4.01**
Error c	18	0.006	0.006	0.054	0.047	5.01	8.02	4.15	2.7	0.0004	0.0006	0.4	0.26
T	53	–	–	–	–	–	–	–	–	–	–	–	–
CV (%)	–	2.81	2.86	3.6	3.89	5.19	6.12	2.8	2.25	3.4	3.98	7.2	5.3

[a] ns, *, and ** indicate non-significance, significance at 1% probability level, and significance at 5% probability level, respectively

gen from urea fertilizer source) was given to the main land manually in two stages of maximum tillering and beginning of flowering according to the treatments. In the stage of physiological ripening, four 2-meter lines were removed from the middle of each plot. To measure the total amount of dry matter, three plants were removed from each plot and kept in a 75 °C oven for 72 h and then weighed. The harvest index was calculated via Eq. 1:

$$HI = \frac{\text{grain yield}}{\text{straw yield and grain yield}} \times 100 \quad (1)$$

Equation 2 was used to calculate water-use efficiency:

$$\text{Water use efficiency} = \frac{\text{grain yield}}{\text{water used}} \quad (2)$$

To measure characteristics such as leaf relative water content and the leaf free proline content, samples were collected in the flowering stage. To measure early-morning leaf relative water content, completely developed leaves were selected and punctured, immediately after harvesting, in disks of relatively equal size and then weighed, the value of which was recorded as the fresh weight (FW). To measure the turgid weight of leaf disks, they were submerged in water and placed in the dark for 48 h and then dried with drier sheets and their turgid weight (TW) was measured with a scale. Afterward, they were placed in a 75 °C oven for 48 h until the dry weight (DW) value reached a constant, and then RWC was calculated via Eq. 3 (Wheatherley 1973).

$$RWC\ (\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100 \quad (3)$$

The method of Bates et al. (1973) was used to extract and assess the leaf free proline content. To do so, 50 mg of fresh leaf tissue was ground using 4 mL of 3% sulfosalicylic acid solution and then centrifuged at 13,000 rpm (revolutions per minute) for 20 min. Then, 2 mL of the obtained supernatant was dissolved in 2 mL of glacial acetic acid and 2 mL of ninhydrin acid (including 1.25 mg ninhydrin, dissolved in 20 mL of 6-molar phosphoric acid and 30 mL of glacial acetic acid) at 100 °C for 1 h, and then immediately transferred to an ice bath. Afterward, 4 mL of toluene was added to the reaction solution and its absorption (color phase) was read at 520 nm. Finally, the proline level was calculated based on micrograms of proline per gram of fresh weight.

The analysis of variance and statistical calculations were carried out via SAS software version 9.1 and the mean value of the studied characteristics were compared using Duncan's test at a 5% probability level. The Excel 2013 program was used to illustrate the graphs.

Results and Discussions

Grain Yield and Water-use Efficiency

The effect of irrigation × fertilization × cultivar interaction on grain yield was significant in both years (Table 3). Reduced water consumption was observed in 2017 for I2 (19.72% in the Gilaneh cultivar and 16.61% in the Hashemi cultivar) and I3 (43.48% in the Gilaneh cultivar and 41.4% in the Hashemi cultivar), as compared to flood irrigation. Reduced water consumption was also observed in 2018

Table 4 Comparison of means for the irrigation interval × nitrogen fertilizer × cultivar interaction effect on the studied characteristic

	Dry matter (ton ha^{-1})		HI (%)		RWC (%)		WUE (Kg m^{-3})		Proline μg. g^{-1}FW	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
I1N1C1	6.5cde[a]	6.6b	38.8efg	33.48g	65.3e	66.1f	0.458h	0.38k	6.11i	6.61fe
I1N1C2	5.8f	5.5cd	36.87fgh	34.69fg	70.7d	71.5e	0.328j	0.3o	7.91fgh	6.29f
I1N2C1	7.6a	7.6a	42.78def	40.79efg	79.1bc	80.1bc	0.596f	0.56f	7.49ghi	7.1ef
I1N2C2	6.9bcd	6.9b	43.61de	44.15bcde	80.9bc	82.8b	0.487g	0.47g	8.07fgh	8.82ef
I1N3C1	7.6a	8.03a	51.29ab	53.23a	87.1a	85.8a	0.732d	0.74d	7.55gh	6.31f
I1N3C2	7.3ab	7.96a	44.4cde	50.91abc	87.9a	88.2a	0.584f	0.66e	8.156fgh	6.66ef
I2N1C1	5g	5.1de	40.05efg	40.4efg	60.3f	59.9g	0.46gh	0.47h	8.15fgh	7.38ef
I2N1C2	4.4g	4.8e	42.78def	47.41abcde	63.1ef	63.8f	0.405i	0.41jk	8.09fgh	8.12de
I2N2C1	6.3def	6.8b	32.4h	34.96fg	72.2d	72.07e	0.492g	0.46i	8.9efg	9.52cd
I2N2C2	5.8f	5.9c	50.77abc	50.6abc	77.6c	79.4c	0.571f	0.56f	10.7cd	10.64bc
I2N3C1	7.1abc	7.7a	47.09bcd	50.58abc	81.2b	81.1bc	0.805c	0.83c	9.8de	9.8c
I2N3C2	6.1ef	6.9b	52.79ab	49.05abcd	81.4b	81.5bc	0.684e	0.58f	11.47c	10.15c
I3N1C1	4.6g	4.7ef	39.22efg	41.69def	54.0g	51.5h	0.583f	0.59f	7.51ghi	6.63ef
I3N1C2	3.4h	4.1f	36.6gh	45.29bcde	54.1g	60.4dg	0.419i	0.42j	6.48hi	6.5f
I3N2C1	5g	5.9c	36.63gh	43.6cde	72.4d	72.7e	0.7de	0.76d	9.25ef	9.67c
I3N2C2	5.9ef	5.8c	51.21ab	50.62abc	72.2d	74.4de	0.838c	0.9b	13.22b	11.84b
I3N3C1	6.8bcd	6.6b	47.72bcd	48.97abcd	72.8d	76.3d	0.99a	1.02a	16.56a	16.18a
I3N3C2	5.9ef	5.8c	54.56a	51.53ab	77.7c	74.4de	0.872b	0.81c	16.48a	15.4a

[a] In each column, means with at least one similar letter are not significantly different according to the LSD test ($p \le 0.05$)

Table 5 Correlation coefficients of the studied characteristic

	Yield 1	Dry matter 2	HI 3	RWC 4	WUE 5	Proline content 6
1	1	–	–	–	–	–
2	0.85**	1	–	–	–	–
3	0.75**	0.33	1	–	–	–
4	0.89**	0.81**	0.59*	1	–	–
5	0.6*	0.39	0.65**	0.3	1	–
6	0.35	0.16	0.46	0.22	0.72**	1

* and **: Significant correlation at the 5 and 1% levels of probability, respectively

for I2 (17.45% in the Gilaneh cultivar and 13.94% in the Hashemi cultivar) and I3 (42.3% in the Gilaneh cultivar and 43.7% in the Hashemi cultivar), as compared to flood irrigation (Table 4). These differences may be resultant from the different levels of precipitation during the two studied years (Table 1). Along with the reduction of water consumption, water-use efficiency also showed an increase such that this characteristic was at it its maximum value in the I3 treatment (Table 5).

Changes in grain yield response to reduced water consumption was dissimilar in different cultivars and different levels of N fertilizer. When applying 50 and 100 kg/ha of fertilizer in the Hashemi cultivar, switching from I1 to I2 wasn't accompanied by reduced grain yield and increased water-use efficiency. However, when 75 kg/ha of fertilizer was applied, the yield was similar in all three irrigation regimes, whereas water-use efficiency increased significantly in I2 and I3, as compared to I1 In the Gilaneh cultivar, grain yield of I2 and I3 in all three fertilizer levels was lower than that of flood irrigation (Fig. 1). It appears compared to the Hashemi cultivar, the yield of the Gilaneh cultivar is more sensitive to AWDI and reduced levels of consumptive water, and the increased water-use efficiency in this cultivar is merely due to reduced water consumption and not the result of increase or consistency in grain yield.

Investigating the effect of increased irrigation interval on changes in the yield of the Hashemi cultivar, as the cultivar constituting the highest rate of area under cultivation in northern Iran, Razaei et al. (2009) reported that the 5-day and 8-day irrigation intervals reduced water consumption in this cultivar at an average of 40 and 60%, respectively, as compared to flood irrigation, though the change in yield was not statistically significant, but implementing the 11-day irrigation interval caused a significant reduction in yield. Previous research studies have reported that, compared to flood irrigation, the AWDI method results in increased water-use efficiency, growth, and yield (Razaei et al. 2009; Lampayan et al. 2015). However, in the present study, the changes in yield were dependent on cultivar and the amount of applied nitrogen fertilizer. The effects of this irrigation method on grain yield may be related to soil type, the degree of soil dryness, time of irrigation, weather conditions during the growing period, N fertilizer management, and the type of grown cultivar (Islam et al. 2018; Yao et al. 2012).

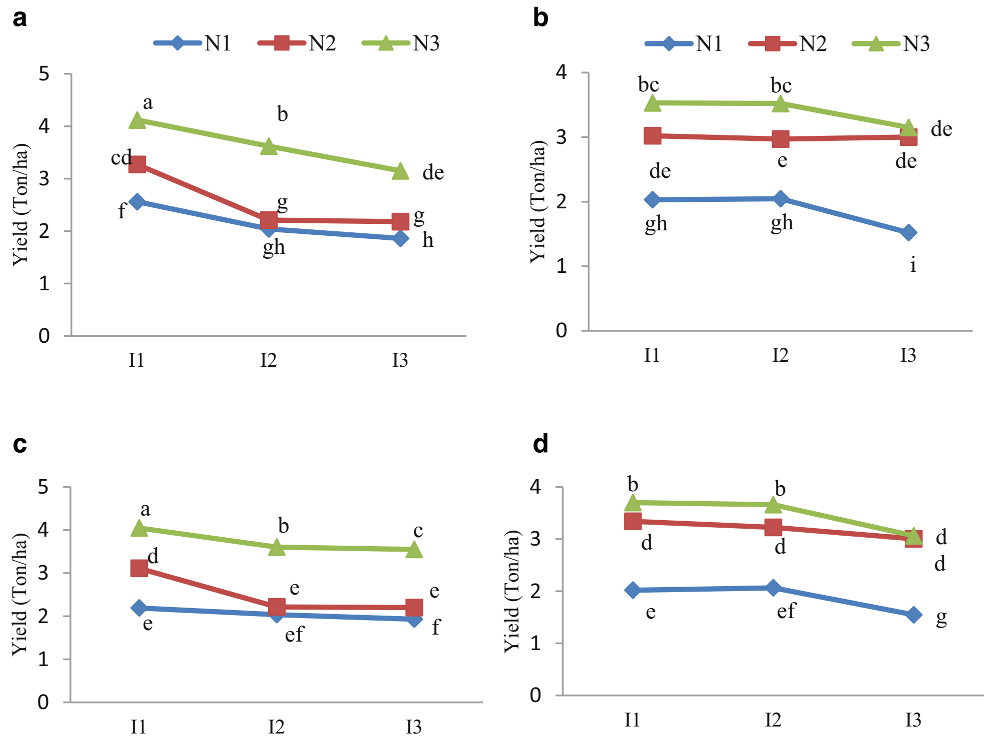

Fig. 1 Effect of irrigation × nitrogen fertilizer × cultivar interaction on grain yield. **a** Gilaneh cultivar grain yield in 2017; **b** Hashemi cultivar grain yield in 2017; **c** Gilaneh cultivar grain yield in 2018; **b** Hashemi cultivar grain yield in 2017

Total Dry Matter and Harvest Index

The irrigation × fertilization × cultivar interaction effect on total dry matter and the harvest index was significant for both years (Table 3). Switching from flood irrigation to AWD irrigation along with decreased consumptive water significantly reduced the total amount of produced dry matter in both cultivars. In contrast to the results of the present study, Ye et al. (2013) stated that total biomass in the harvesting stage was higher in AWDI than in flood irrigation and ascribed this increased biomass as the result of advanced root growth. However, in the present study, increasing the irrigation interval reduced dry matter by 12 to 22% in 2017 and 16 to 24% in 2018, as compared to flood irrigation (Table 4). The total dry matter and the harvest index responded to the application of N fertilizer in all three irrigation levels and showed an upward increase, consistent with the increase of fertilizer application (Table 4). Increasing the application of nitrogen fertilizer from 50 to 75 kg/ha and from 75 to 100 kg/ha increased the harvest index by approximately 8% and 13%, respectively. The harvest index in 2018 was lower than that of 2017 (Table 4).

Maximum total dry matter and harvest index in both cultivars were observed in the N fertilization treatment of 100 kg/ha. In the I1 level, a greater difference in yield and total dry matter was observed among the various levels of fertilizer in both cultivars, but by increasing the irrigation interval, less difference in yield and total dry matter was observed among various fertilizer levels. These results are consistent with those of Niang et al. (2017), which stated that the response of rice to N fertilizer application in the conditions of water availability is greater than when the plant is faced with water limitations. A study conducted in China has reported that AWDI results in total mass, grain yield, water-use efficiency, and nitrogen-use efficiency comparable to flood irrigation, and when 240 kg/ha of nitrogen fertilizer was tested alongside this system of irrigation, these characteristics showed a significant increase for AWDI, in comparison to flood irrigation (Ye et al. 2013).

Leaf Relative Water Content and Leaf Proline Content

The increase of leaf proline content and the reduction of leaf relative water content, consistent with increased irrigation interval, were observed in 2017 and 2018. Fertilization also increased this characteristic in the cultivars. The reduction of the leaf relative water content and the increase of the leaf proline content, in response to increased irrigation interval, has also been previously reported by Kunjammal et al. (2020) and Sureshkumar and Pandian (2017). The difference between the leaf water content in the two cultivars depended on the irrigation level and fertilizer treatment where the Hashemi cultivar excelled regarding the I1 level and 50 kg/ha fertilization treatment, while in the 75 and 100 kg/ha fertilization treatments both cultivars were similar in this regard. The Hashemi cultivar was superior in the I2 level and application of 50 and 75 kg/ha fertilizer, and the Gilaneh and Hashemi cultivars had similar leaf relative water content in the 100 kg/ha level of fertilizer. The two cultivars had similar leaf relative water content in the I3 level and the 50 and 75 kg/ha fertilization treatments, whereas in the 100 kg/ha fertilization treatment, the Hashemi cultivar was superior regarding this characteristic. However, in 2018 the difference between cultivars was negligible in the N3 level, and in the N1 and N2 levels in all irrigation treatments, the Hashemi cultivar showed higher leaf relative water content (Table 4). In the I1 level, there was less difference of leaf proline content among the fertilization treatments, but in the I2 level, the leaf proline content increased among the fertilization treatments and applying 100 kg/ha N fertilizer had the highest impact on leaf proline content, with this difference being tremendous in the I3 level. A comparison between cultivars shows that in the flood irrigation, the leaf proline content was relatively similar in both cultivars, but by increasing the irrigation interval, the Hashemi cultivar showed more increase in leaf proline content (Table 4).

The effect of nitrogen fertilizer on the increase of RWC in rice has been previously reported by Rashid et al. (2016) reporting that changing the level of N fertilizer application from 0 to 100 kg/ha increases RWC in rice The greater leaf relative water content in the Hashemi cultivar, compared to the Gilaneh cultivar could be due to the higher leaf proline content of this cultivar (Table 4). Consistent with the results of the present study, a study on four cultivars, i.e. Vandana, IR36, IR72, and Swarna, reported that the Vandana and Swarna cultivars exhibited higher leaf proline content and better osmoregulation and RWC (Dasgupta et al. 2015). As an osmoregulator, proline has the ability to maintain RWC at a high level in drought-resistant cultivars, assisting in the reduction of cells' osmotic potential without the actual reduction of leaf water content and giving the roots the ability to absorb more water (Blum 2005). In the present study, the leaf relative water content showed the highest correlation with grain yield ($R^2 = 0.89$) and total dry matter ($R^2 = 0.81$) (Table 3).

Conclusion

The Gilaneh cultivar has shown more sensitivity to increased irrigation interval than the Hashemi cultivar. Compared to flood irrigation, alternate wetting and drying irrigation caused yield decline in the Gilaneh cultivar, while the Hashemi cultivar showed a similar yield in the 7-day irrigation interval in all three levels of fertilization. This level of grain yield in this treatment was accompanied by less water consumption and higher water-use efficiency. This cultivar also showed higher leaf relative water content and higher proline content, compared to the Hashemi cultivar. Applying 100 kg/ha of nitrogen fertilizer exhibited higher grain yield, total dry matter, and harvest index in all three irrigation treatments. This treatment also showed greater water-use efficiency compared to the other two levels of fertilization. According to the results of the present study, it appears that in the circumstances of water limitation, farmers of the region can consume less water by implementing the alternate wetting and drying irrigation method, selecting the right cultivar, and managing the application of nitrogen fertilizer, practices through which yield does not undergo any changes or at least shows less reduction.

Conflict of interest S. Eisapour Nakhjiri, M. Ashouri, S.M. Sadeghi, N. Mohammadin Roshan and M. Rezaei declare that they have no competing interests.

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